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# EFFECT OF RYE AND VETCH GREEN MANURES ON THE MICROFLORA, NITRATES, AND HYDROGEN-ION CONCENTRATION OF TWO ACID AND NEUTRALIZED SOILS [1]

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## INTRODUCTION

The depletion of the organic matter and available plant food in soil through certain systems of farming has created a great interest in methods of increasing or even maintaining the fertility of cultivated soils. Animal manure seems to be one of the most effective materials, and its use has always been recommended. However, in this age of motorized transportation manure in large quantities is often difficult to obtain. Aside from the use of animal manure the principal means of augmenting the organic matter in soil is through the use of the so-called green manures. Much work on this subject has been done both in this country and abroad. Most of the literature (*33*) [3] deals with the effect of green manuring upon succeeding crops, the humus content, water-holding capacity, and nitrate content of the soil; the relative rate of decomposition of various plants or parts of plants; the availability of the nitrogen in those plants; or the decomposition of plants of different ages.

Microbiological studies of green manuring, excluding its influence upon nitrification, have not been very numerous or very thorough. Plate counts of soil microorganisms were often made several weeks after the application of the green manure, or the green manure was dried, ground up, thoroughly mixed with the soil, and incubated in small containers. For these reasons there seemed to be a need for closely controlled experiments on the microbiology of decomposing green manures in neutral soils and also in the acid and alkaline soils as found in many of the agricultural districts of the country. Such studies could not be carried out in the field, as they would be subject to climatic variations of temperature and moisture. The alternative seemed to be to transport large quantities of field soil into greenhouses, where those two variables might be controlled. One might say that nowhere in field practice would one find these optimum conditions for any great length of time. This is quite true. However, if what happens under optimum conditions is known it might be of assistance in interpreting results obtained under the variable conditions existing in field experimentations.

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[3] Reference is made by number (italic) to Literature Cited p. 121.

Since a great many of the eastern soils are naturally acid, it was elected to study the decomposition of green manures in two of these soils which are located fairly close to Washington, D. C., and upon which considerable agronomic and chemical experimental work had been done both by the Maryland Agricultural Experiment Station and the United States Department of Agriculture. The work was begun in the fall of 1927 and finished in the fall of 1928.

## MATERIALS AND METHODS

### SOILS AND MANURES

The two acid soils selected were of divergent physical character. The fine-textured soil, a Leonardtown clay loam with a pH value of 4.6, was obtained from an uncultivated area on the experimental tract of the Maryland Agricultural Experiment Station located about 3 miles from Leonardtown, Md. The coarser textured soil, a Collington fine sandy loam with a pH value of 5.2, was obtained from an uncultivated area on the experimental tract of the United States Department of Agriculture near Upper Marlboro, Md.

A sufficient amount of each soil was secured to fill two benches in a greenhouse on the Arlington Experiment Farm, Rosslyn, Va. Each bench was divided into 14 sections 3 feet square. Each section had a waterproof cement bottom provided with a drain and wooden sides 12 inches high. The soil after thorough mixing was placed in the sections to a depth of 10 inches and watered with distilled water. It was then kept at the optimum moisture content throughout the experiments.

Sufficient finely ground limestone (100 mesh) was thoroughly mixed with the soil in the even-numbered plots in each series to bring the soil to approximately a neutral reaction. This required a rate of 5 tons per acre for the Leonardtown clay loam and 2.5 tons per acre for the Collington fine sandy loam. (Table 10.) Analysis of the limestone showed an equivalent of 91 per cent $CaCO_3$.

The first experiment consisted in turning under green rye as a green manure on a limed and an unlimed plot of each soil. The rye grown on a separate plot in the greenhouse to a height of 15 inches was pulled up by the roots, washed free from soil, and turned under in a layer about 5 inches below the surface of the soil. The rye was therefore in a succulent condition, consisting largely of leafy material. Each plot received 2,200 gm. of green rye, a rate of 23,476 pounds per acre. The air-dry weight of the rye was 25 per cent of the green weight and the nitrogen content was 3.33 per cent. The rate of application was therefore 5,869 pounds of dry material, containing approximately 200 pounds of nitrogen per acre.

Soil samples consisted of a number of cores of soil taken with a sampling tube 1¼ inches in diameter to a depth of 6 inches. Samples were taken just before the green material was turned under and subsequently 2, 4, 7, 14, 21, 35, 56, 84, and 119 days after it was turned under. Plate counts of total number of microorganisms, fungi, actinomycetes, and determinations of moisture, of nitrates, and of soil reaction were made of each sample.

In the second experiment vetch was substituted for rye as a green manure on some additional plots of the two soils. It was applied in the same manner as the rye, at the rate of 5,000 gm. of green material

per plot, equivalent to an application of 53,355 pounds per acre. The dry weight of the vetch was 27 per cent of the weight of the green material and the nitrogen content was 2.5 per cent. This is equivalent to an application of 14,406 pounds of air-dry vetch and 360 pounds of nitrogen per acre.

Analyses were made as in the first experiment, samples being taken in a similar manner 0, 2, 4, 7, 14, 21, 35, and 56 days after the green manure was turned under.

The treatment of the plots may be summarized as follows:

| Plot No. | Treatment | Plot No. | Treatment |
|---|---|---|---|
| 1 | Control. | 5 | Control. |
| 2 | Limestone only. | 6 | Limestone only. |
| 3 | Rye green manure. | 7 | Vetch green manure. |
| 4 | Rye green manure and limestone. | 8 | Vetch green manure and limestone. |

In order to confirm the results obtained on the first two experiments, the green-manure treatments were duplicated on the same plots in the fall of 1928, the plots having been kept fallow all summer at optimum moisture content. Both the rye and the vetch in this experiment were grown on plots equal in size to those treated, but were located in another bench. The amount of green manure applied, therefore, was determined by the yield of rye or vetch produced on these other plots. The amounts applied are shown in Table 1.

TABLE 1.—*Amount of green manure and its nitrogen equivalent applied to two arid soils*

| Plot No. | Green manure | Collington fine sandy loam | | | | | Leonardtown clay loam | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Green weight | Equivalent dry weight | Equivalent dry weight per acre | Nitrogen | Nitrogen per acre | Green weight | Equivalent dry weight | Equivalent dry weight per acre | Nitrogen | Nitrogen per acre |
| | | *Grams* | *Grams* | *Pounds* | *Per cent* | *Pounds* | *Grams* | *Grams* | *Pounds* | *Per cent* | *Pounds* |
| 3 | Rye | 2,450 | 764 | 8,145 | 1.93 | 157 | 1,020 | 318 | 3,390 | 1.93 | 65 |
| 4 | Rye * | 2,560 | 800 | 8,530 | 1.93 | 166 | 1,430 | 446 | 4,750 | 1.93 | 92 |
| 7 | Vetch | 1,670 | 554 | 5,900 | 2.04 | 120 | 1,140 | 378 | 4,000 | 2.04 | 82 |
| 8 | Vetch * | 2,050 | 680 | 7,250 | 2.04 | 148 | 1,470 | 488 | 5,200 | 2.04 | 108 |

* Limed duplicate.

Soil samples were taken 0, 2, 4, 7, 14, 21, 28, 42, and 63 days after the green manure was turned under, and analyses were made as in the first experiment.

Conditions in the greenhouse with the higher temperature and optimum moisture are quite different from those in the field. For this reason it seemed advisable to include a field test along with the first experiment. In this test both rye and vetch were used as green manures. The vetch was from the same lot as that used in the greenhouse and was applied at the same rate. The air-dry weight of the rye used was 20.7 per cent of the green weight and the nitrogen contained was 2.39 per cent. This application was equivalent to 4,860 pounds of dry matter and 116 pounds of nitrogen per acre. The amount of

nitrogen added in this case was considerably lower than that added in the vetch or that in the rye of the first greenhouse experiments. Treatments were as follows:

| Plot No. | Treatment | Plot No. | Treatment |
|---|---|---|---|
| 1 | Rye green manure. | 3 | Vetch green manure. |
| 2 | Control. | | |

The soil in this field experiment was similar in texture to the Leonardtown soil. The pH value was 5.5.

### PLATING METHODS

As soon as the soil samples arrived in the laboratory they were put through a 10-mesh sieve and thoroughly mixed. Aliquots were then weighed out for determination of the microflora, nitric nitrogen, and moisture.

Dilutions of the soil samples for plating were made in the following manner. To 20 gm. of soil in a sterile mortar enough sterile tap water was added from the dilution flask to make a thin paste. After triturating until the paste was smooth the remainder of the 500 ml. of sterile water was added, mixed thoroughly, and poured back into the dilution flask. This flask was then stoppered and shaken several times over a period of 10 to 15 minutes. Higher dilutions were made by adding 10 ml. to 90 ml. sterile tap water, the final dilution being 1 to 2,500,000. For the total plate counts, 5 ml. of this dilution were pipetted into each of 10 Petri dishes, 120 by 15 mm. In cases where the number of colonies developing on the plate exceeded 200, only 2.5 ml. were used. The actual dilutions were therefore 1 to 500,000 and 1 to 1,000,000, respectively. For the counts of the actinomycetes, 1 ml. of the 1 to 250,000 dilution was pipetted into each of 10 plates, and for the fungi counts, 5 ml. of the 1 to 25,000 dilution.

The medium used in pouring the plates for the total counts was soil-extract agar, which has been shown to favor the growth of a greater number of soil bacteria than any other commonly used medium (*35*). The composition of the soil-extract medium is as follows: Soil extract, 1,000 ml.; $K_2HPO_4$, 0.5 gm.; and agar, 10 gm. If not pH 6.8, reaction should be adjusted to this. The soil extract is made by autoclaving 1,500 gm. of air-dry field soil of ordinary fertility with 4.5 liters of tap water for one-half hour and filtering clear through paper. The final volume of soil extract is approximately twice the original amount of soil. Large quantities may be prepared, put into large flasks, and sterilized for future use.

The plates for the total counts were incubated for three weeks at 28° C. The results given are the average of 10 plates, the number of organisms per gram of soil being calculated by multiplying the average number per plate by the dilution and correcting the result for the moisture content at the time of sampling.

The medium used in pouring the plates for the actinomycetes consisted of soil extract to which was added 0.1 per cent $K_2HPO_4$, 0.1 per cent $NaNO_3$, 1 per cent glycerin, and 1 per cent agar. The reaction was adjusted to pH 6.8. The incubation period for the plates was 10 days at 28° C.

For the estimation of the fungi an acid medium of the following composition was used: Soil extract, 1,000 ml.; $KH_2PO_4$, 1 gm.; $NaNO_3$, 1 gm.; agar, 25 gm., and dextrose, 10 gm. The dextrose is added after melting and the reaction adjusted to pH 4.2 by adding equal quantities of N HCl and N $H_2SO_4$. This usually requires about 1 ml. of each. Care in sterilizing this highly acid medium must be exercised or the agar will be broken down. It has been found safe both as regards the solidifying power and the sterility of the medium to autoclave at 12 pounds pressure for 12 minutes provided the medium is in a water bath during the heating. The plates were incubated for three days at 28° C.

## LITERATURE REVIEW

The literature dealing with the effects upon the numbers of bacteria of neutralizing the soil acidity with limestone or other forms of lime is quite voluminous. While no attempt will be made in this paper to cover it, it may be advisable to review briefly some of the work along this line.

Chester (*15*), Fabricius and Von Feilitzen (*18*), and Fischer (*19*) were among the first to note that lime increased the number of bacteria. Brown (*10*, *11*) found that lime increased the plate counts of soil in pots in the greenhouses and also those of field soils. Beckwith, Vass, and Robinson (*6*) studied the effect of lime on soils of varying reaction and found that lime increased the number of bacteria only in the acid soils. In one acid soil high in organic matter, while increasing the number of bacteria, lime decreased the number of molds.

Bear (*5*), applying various amounts of $CaCO_3$ to soil, reported increases in the number of bacteria up to the heaviest application (40,000 pounds) 12 weeks after treatment. Fulmer (*21*) found that calcium carbonate, magnesium carbonate, and limestone increased the bacteria in two acid soils, magnesium carbonate producing the greatest effect. Noyes and Connor (*30*) found that calcium carbonate increased the bacterial contents of 5 typical acid soils 10 months after application in the greenhouse and that the increase was due to aerobic bacteria.

Waksman (*38*) found that lime added to an acid sandy loam not very rich in organic matter increased the bacteria and actinomycetes and decreased the fungi.

Barnette (*4*) used limestone and other forms of calcium and found a rapid increase in total plate counts for two weeks, which was followed by a decrease to nearly a constant level at four weeks. Chatterjee (*14*) also reported an increase in total counts due to liming.

The effect of turning under green manures upon the total number of microorganisms has been studied to a less extent. Engberding (*17*) added both green rye and vetch to soil and found that the plate counts of the microorganisms had increased at the end of 7 weeks from 17,000,000 to 100,000,000 with the rye and to 80,000,000 with the vetch. After 10 weeks they had dropped to 78,000,000 and 60,000,000, respectively, and the checks had increased to 30,000,000. Brown (*12*), on the other hand, reported that green manuring with rye, cowpeas, and clover did not always increase the number of bacteria in field soil. This may have been partly due to the low moisture content of the soil. In a 2-year rotation (*13*), turning under rye increased the plate counts over those of the regular rotations.

Briscoe and Harned (*7*, *8*), using green alfalfa, oats, and cowpeas for green manure, found a direct relationship between the total plate counts of soil microorganisms and the amount of organic matter added. How long the materials were allowed to decompose before the samples were taken was not stated. Furthermore, different kinds of plating media were used during the experiments.

Hill (*23*) obtained a great increase in the number of bacteria in various soils treated with bluegrass, clover, and alfalfa green manures. Clover, especially, increased the numbers at four weeks, the numbers decreasing after that.

Baldwin and Smith (*3*) applied green rye at the rate of 12 tons per acre and found after one month an increase in the number of bacteria growing upon beef agar. After three months the treated soil contained approximately the same number as the controls.

Martin (*27*) added rye, oats, and buckwheat as green manure and allowed it to decompose 12 months before sampling. Realizing

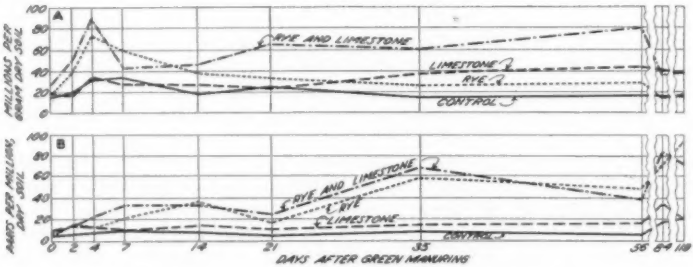


FIGURE 1.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Collington fine sandy loam as affected by different treatments; rye green-manure series

that the interval was too long, he ran another series and took samples at the end of 4 months. In this series no control was analyzed. In general, total counts were higher in the green-manured soil.

## EFFECT OF TREATMENTS ON THE TOTAL PLATE COUNTS OF THE SOIL MICROFLORA

It will be recalled that there were three treatments for each acid soil and each green manure, viz—(1) limestone, (2) rye or vetch, and (3) limestone and rye or vetch. Also certain conditions of the experiments should be emphasized, viz, the application of the whole green plant (roots and tops), the uniform depth of 5 inches at which the green material was turned under, the constant temperature and moisture, and the frequent samplings during the early stages of decomposition of the green manure.

The total counts made on the four plots of the Collington fine sandy loam of the rye green-manuring series are shown in Figure 1. The untreated control shows some variation in numbers during the course of the experiment, but as a whole the counts are fairly uniform. The numbers in the limed plot followed those in the control until the thirty-fifth day, when there was an increase to nearly 40,000,000, this figure persisting to the end.

When the green rye was turned under a rapid increase in the number of microorganisms took place both on the limed and on the unlimed plots. In 4 days the number increased to 74,000,000 where rye alone was added and to 90,000,000 with rye and limestone. A rapid decrease followed, which in the latter case was followed by another increase, reaching a peak at 80,000,000 in 56 days. The acid rye-manured plot, on the other hand, shows a gradual decrease in total counts from the fourth to the thirty-fifth day, the numbers remaining only slightly above the control until the end of the experiment, when they were practically identical. At the end the plate counts of the two limed plots reached the same level in spite of the addition of rye to one of them, and this level was well above that of the other two plots which had no limestone.

The results of plating out the Leonardtown series are given in Figure 2. The control, as in the Collington series, shows only a

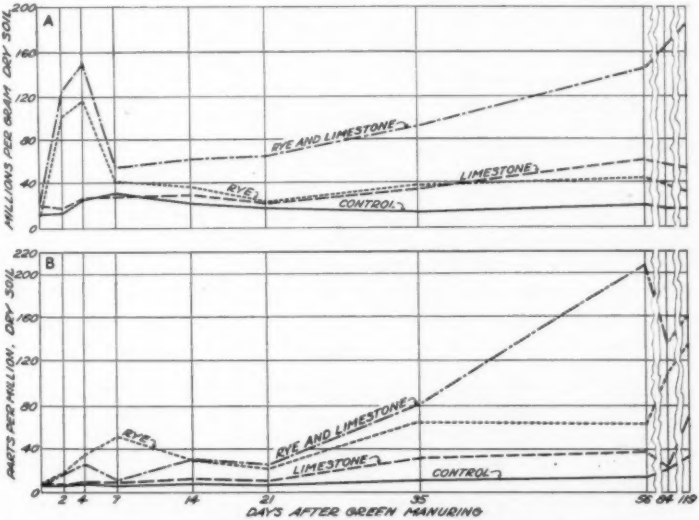


FIGURE 2.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Leonardtown clay loam as affected by different treatments; rye green-manure series

slight change in the number of microorganisms. The limed control shows an increase at the 35-day sampling and a further increase to 60,000,000 in 56 days, the count falling off some at the end. The application of green rye caused an increase to 116,000,000 in 4 days, whereas rye and limestone together gave a peak of 151,000,000 at that time. In both cases there was a sharp drop at the 7-day period to 41,000,000 and 56,000,000, respectively. Thereafter very little change took place in the acid rye-manured plot. But with the rye and limestone together there was a steady rise in the plate counts to the fifty-sixth-day sampling and then a jump to 184,000,000 at 119 days.

In the next experiment the effect of turning under green vetch was studied under similar conditions, with the exception of an irregularity in limestone treatment, which will be discussed later.

Figure 3 shows the relative number of microorganisms found in the four plots of Collington soil of the vetch green-manure series. In the control and limed plots the numbers are similar to those found in the rye series. Vetch in the acid soil affected the organisms much the same as the rye, except that the peak was not reached until the seventh day. In the case of the vetch and limestone the great increase

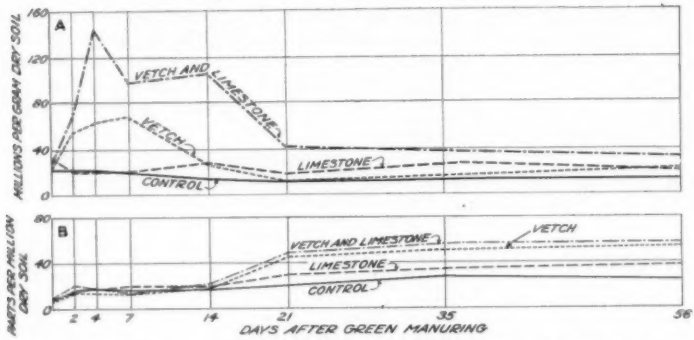


FIGURE 3.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Collington fine sandy loam as affected by different treatments; vetch green-manure series

occurred at four days. At seven days there was a considerable decrease but not until the twenty-first day was it pronounced. There was no secondary increase as found in the rye series.

Plate counts of the four plots of Leonardtown clay loam of the vetch green-manuring series are shown in Figure 4. Here again

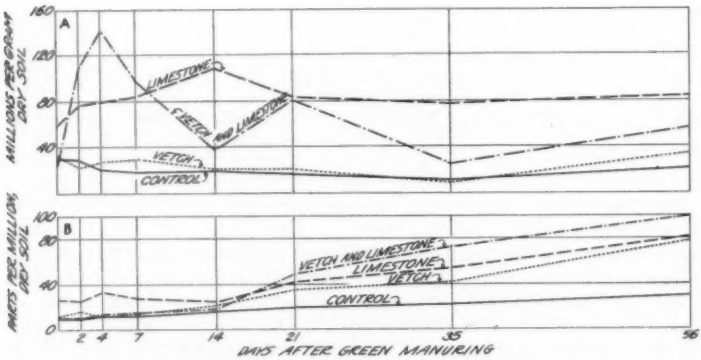


FIGURE 4.—Comparison of total plate count (A) and nitrate nitrogen (B) in Leonardtown clay loam as affected by different treatments; vetch green-manure series

the numbers in the control are fairly consistent. Where limestone and vetch were added there is the same rapid increase in numbers in four days, followed by a drop to relatively near the level at the start. Further comparison with Figure 2 reveals only differences which seem to be inexplicable. For instance, the addition of vetch to the acid soil failed to affect the total counts at any time, the

counts remaining very close to those of the control plot. The high numbers found in the limed plot during the whole period may be partially explained by the fact that the limestone was added to this soil 10 weeks before it was added to the plot receiving the vetch. Dorsey (*16*) has shown that limestone reduces the acidity gradually for 6 to 8 weeks. Karraker (*26*) found that the effect of limestone in bringing about an increased growth of alfalfa on acid soils was delayed from a few weeks to two months. This delayed action of limestone may be the cause of the large numbers in the limed plot. However, the corresponding plot of the Collington soil which was also limed

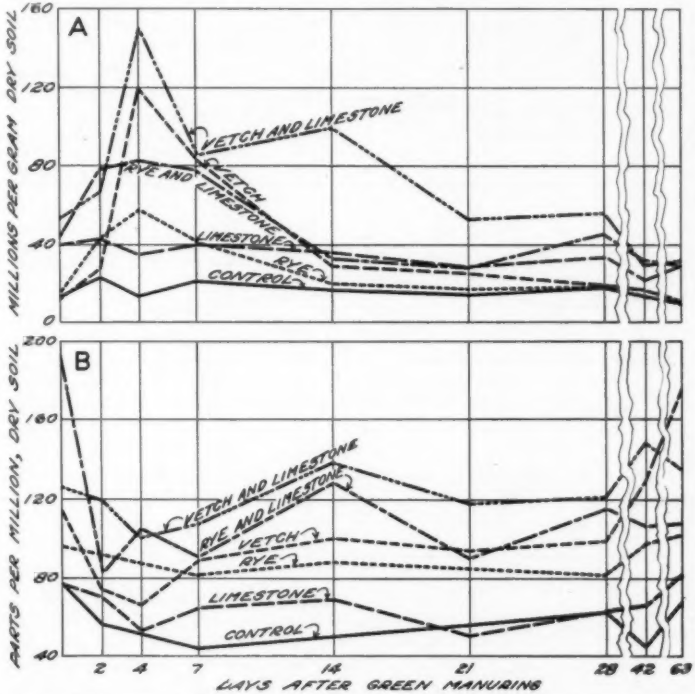


FIGURE 5.—Comparison of total plate count (A) and nitrate nitrogen (B) in Collington fine sandy loam as affected by different treatments; second application of rye and vetch green manures

previously did not show this delayed effect, nor did it show the great variations as noted on the Leonardtown limed plot at 4, 7, and 14 days. Of course, these soils are very different physically, and the Leonardtown is more acid. However, Barnette (*4*) observed a rapid increase in plate counts for two weeks due to limestone, after which there was a decline to a constant level at four weeks. Whatever the explanation, the fact remains that the limed control plot contained the greatest number of microorganisms after the seventh-day sampling.

For several reasons it seemed advisable to repeat the green-manure treatment on these same plots. This was done in the fall of 1928, the soil in the meantime being kept moist and fallow.

The results on the Collington fine sandy loam are plotted in Figure 5. The plate counts of the three acid plots were the same at the beginning. On the three limed plots, however, they were more than double, the vetch plot highest, rye next, and limed plot lowest. Comparing the end of Figures 1 and 3 with the beginning of Figure 5, it will be noted that the long fallow period produced practically no

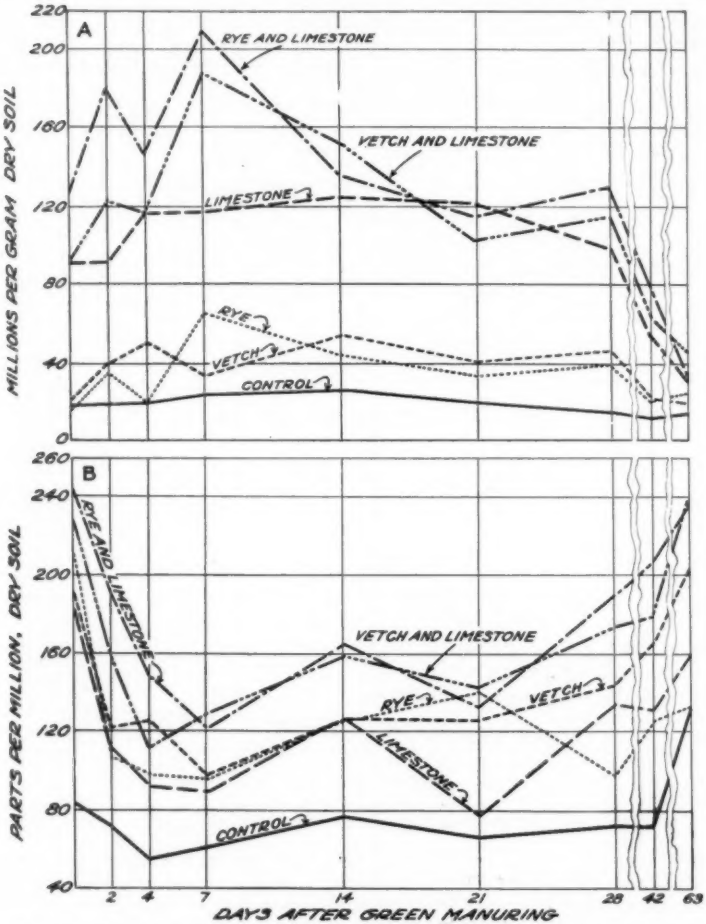


FIGURE 6.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Leonardtown clay loam as affected by different treatments; second application of rye and vetch green manure

change in the total count of the acid plots and only small increases in that of the limed rye and limed vetch plots. Throughout this experiment the plate counts of the control and the limed plots remained fairly uniform. The decomposition of the green manure was exceedingly rapid. The numbers in all cases were very high in four days, especially where vetch was turned under. In both the rye and vetch

acid plots the numbers decreased to the level of the control in 28 days whereas in the limed plots at this time the effect of the green manure was still noticeable, as indicated by a slight secondary rise. Again comparing this figure with Figures 1 and 5, it will be seen that the curves are remarkably similar in spite of the previous treatments.

On the Leonardtown clay loam the effect of the treatments on the number of microorganisms is shown in Figure 6. The addition of green rye to the acid plot caused an increase in number which reached a high point in seven days, a low number, for some unknown reason, being recorded on the fourth day. The usual decrease followed. With vetch turned under, the number of microorganisms increased to 50,000,000 in 4 days, followed by a decrease at 7 days and a subsequent increase to 53,000,000 in 14 days. This was followed by a slight decrease at the next two samplings and quite a definite decrease on the last two sampling dates.

The curves indicating the numbers on the limed plots form a distinct group from the curves of the corresponding unlimed plots.

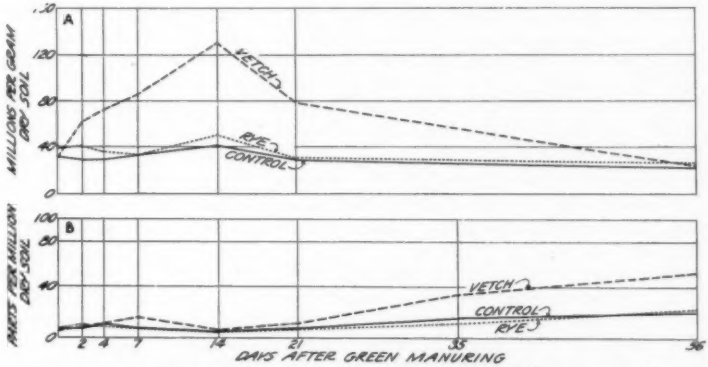


FIGURE 7.—Comparison of total plate counts (A) and nitrate nitrogen (B) in Keysport clay loam as affected by rye and vetch green manuring under field conditions

With limestone alone there were increases for 14 days followed by a slight decrease, which became much larger at 42 and 63 days. The rise in numbers may have been due in part to stirring the soil. All plots were stirred at the same time so as to eliminate any discrepancy which might arise because of the stirring incident to the turning under of the rye and vetch green manures. The limed rye and the limed vetch plots showed the usual rapid increase in numbers to the peak at 7 days, followed by a subsequent decrease. At 63 days the counts of all the limed plots had decreased greatly and were only slightly above those of the unlimed series.

Since the conditions of temperature and moisture were quite different in the greenhouse from what is usually found in the field, a preliminary experiment was made in the field to determine to what extent greenhouse and field results would correlate. The soil for this field experiment was a Keysport clay loam located on Arlington Farm, Virginia. It had been limed some years previously but was slightly acid (pH 5.5).

Both rye and vetch were used as green manures. The total plate counts in the vetch-manured plot (fig. 7) show the characteristic rapid

rise, but instead of a 4-day or 7-day peak the highest count came on the fourteenth day after treatment. The temperature of this field soil was not taken. It is known, however, that it was considerably below that of the soil in the greenhouse which was kept between 20° and 22° C. Moisture was also somewhat lower. These factors undoubtedly slowed up the decomposition and account for the retarded maximum number of organisms.

The rye was field grown and of a slightly different composition than that used in the greenhouse. It affected the number of soil organisms very little, comparatively. No explanation is offered for the failure of the rye to produce an increase in the soil flora in this case.

## EFFECT OF TREATMENTS ON THE BACTERIA

The total plate counts of the microorganisms in soil comprise the bacteria, actinomycetes, and fungi. The bacteria are the most numerous ordinarily. In fact, many workers have considered the total plate count as representing the bacteria and have often reported the plate counts as numbers of bacteria. In this paper the authors have preferred to report the total plate counts as such and to report separately the numbers and kinds of bacteria occurring on those plates as determined by cultural and staining methods.

One of the 10 plates of each soil sample used in making the total counts was selected after the counts had been made and all bacterial colonies picked off and inoculated into a nutrient soil-extract agar.[4] This medium gave a much better growth than the soil-extract agar used for plating and was also better than ordinary nutrient agar for many of the soil organisms. After growth had occurred smears were made and stained by Gram's method as modified by Jensen, reported by Orla-Jensen (*32, p. 25, footnote*). This method has been used by the senior author for 10 years, and while it gives good results no special recommendation is made for it. Although it is well known that bacteria may vary in their reaction to the Gram stain from time to time, this seemed to be the only feasible method of roughly dividing the bacteria into groups.

In order to control the accuracy of the staining method, one set of 63 bacterial cultures was transferred after the smears were made for the Gram staining. These new cultures were subsequently smeared and stained and were found to have the same reactions except in three cases. This is not considered a serious error.

In Figure 8 are shown the numbers of Gram-negative and Gram-positive bacteria found in the Collington soil of the rye green-manuring series. At first there was a tendency for the members of both groups to increase in number. Later, without limestone, both the negative and positive forms decreased and were no more numerous at 21 days than the control. Where rye was added to the limed soil there was a decrease at 7 days, which was followed by increases in both groups. The Gram-negative forms increased much more than the positive forms and were still high in number at 56 days, whereas the positive forms had decreased to the level of the control. Limestone increased the Gram-negative bacteria, the increase beginning at 14 days and

[4] The formula for this medium is as follows: Soil extract, 1,000 ml.; $K_2HPO_4$, 0.5 gm.; ammonium citrate, 0.5 gm.; peptone, 1.0 gm.; beef extract, 0.5 gm.; $NaNO_3$, 0.5 gm.; $MgSO_4$, 0.2 gm.; $CaCl_2$, 0.2 gm.; dextrose, 1 gm.; and agar, 10 gm. Reaction adjusted to pH 6.8 by NaOH.

rising steadily to the end. It produced no effect on the positive group.

The Gram-negative and Gram-positive bacteria in the more acid Leonardtown clay loam (fig. 9) were affected to a greater degree than they were in the Collington soil. The initial increase of both

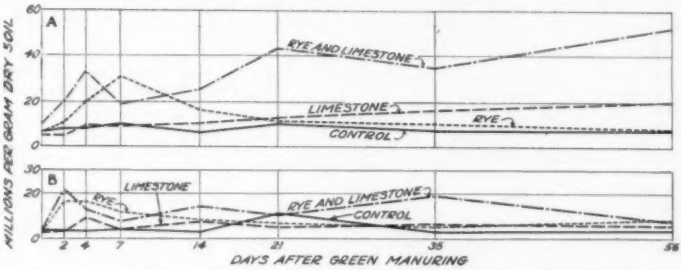


FIGURE 8.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Collington fine sandy loam, as affected by different treatments; rye green-manure series

kinds was very marked, rye alone apparently producing the greatest stimulation of the positive group, whereas rye and limestone most strongly affected the negative group. The sudden fall in the number of both at the 7-day period is very striking and agrees with the fall in the total counts. (Fig. 2.) The positive group increased again somewhat in the case of rye and limestone, but it was not

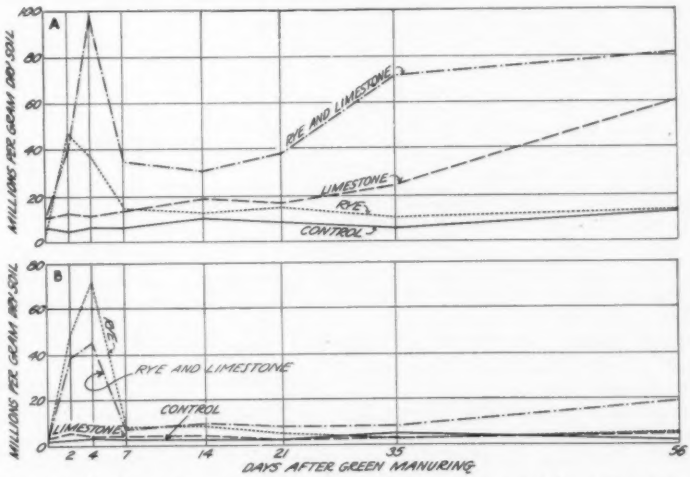


FIGURE 9.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Leonardtown clay loam, as affected by different treatments; rye green-manure series

affected by either the rye or limestone when used separately. On the other hand, the Gram-negative group increased enormously in the limed rye-treated plot and also in the limed plot. In the latter case the effect of the limestone was delayed until the thirty-fifth-day sampling. Comparing this figure again with Figure 2, it is very

evident that the secondary increase in the total plate counts is due to the increase in the bacteria, and that this increase is, to a large extent, due to the effect of the limestone upon the Gram-negative forms.

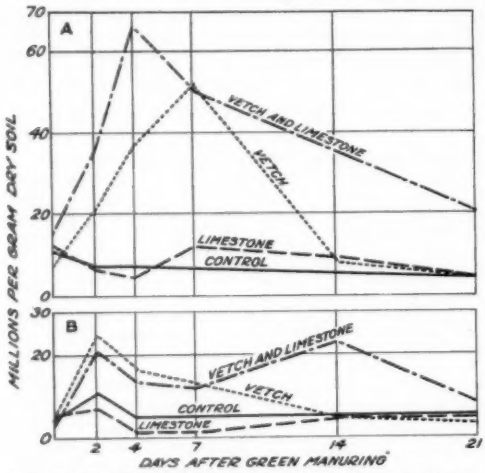


FIGURE 10.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Collington fine sandy loam, as affected by different treatments; vetch green-manure series

In the vetch-manuring experiment on the Collington soil the Gram-negative group (fig. 10) increased much faster and to a greater height than it did when rye was added (fig. 8). With vetch alone the highest number occurred on the seventh day. At 21 days the counts had decreased to those of the control and limed plots. As mentioned above, this limestone plot is not comparable to the one in the rye green-manure series, due to the fact that 10 weeks elapsed between the application of the limestone and the turning under of the vetch. Vetch and limestone increased the negative group to over 66,000,000 in 4 days. Then a sharp decline to 20,000,000 occurred at 21 days.

The Gram-positive group increased 2 days after the vetch was turned under, but the fourth and seventh day samplings showed a decrease. After 14 days this group was no more numerous than the controls. In the limestone-vetch soil there were two maxima (2 and 14 days). The numbers were reduced to practically the same level as the controls at 21 days.

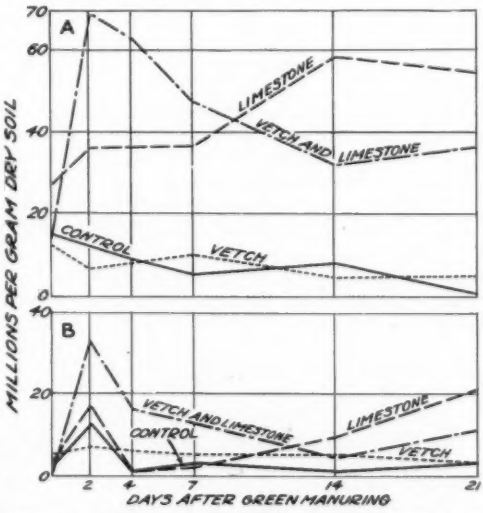


FIGURE 11.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Leonardtown clay loam, as affected by different treatments; vetch green-manure series

The isolation of cultures was stopped with the twenty-first-day sampling because the total plate counts showed no secondary increase as in the first experiment and only a slight decrease after this period.

The turning under of vetch in the Leonardtown soil did not have the same effect as in the Collington, nor was the effect similar to that produced by rye in either soil. This fact was noted in dealing with the total counts and no explanation can be offered for it. For instance, the turning under of vetch in the unlimed soil had no effect upon either the Gram-negative or the Gram-positive bacteria. (Fig. 11.) However, on the limed soil vetch increased both groups at two days as in the other experiments. Thereafter, while these two groups were decreasing on the vetch-limestone plot, they were increasing under the effect of limestone alone.

The effect of rye and vetch green manures upon the Gram-negative and Gram-positive bacteria in Keysport clay loam under field conditions is shown in Figure 12. Both groups of bacteria seem to be equally affected, the highest counts occurring with both green manures at the 14-day sampling. The vetch, as stated above, was from the same lot that was turned under in the greenhouse soil, whereas the rye was field grown and less succulent. This may have had some influence on the development of the bacteria. At least, this figure shows that the increase in the bacteria is slowed up under field conditions but that there is rapid rise in numbers during the first two weeks of the decomposition of vetch. The decomposition of the rye caused only a slight increase at the 14-day sampling.

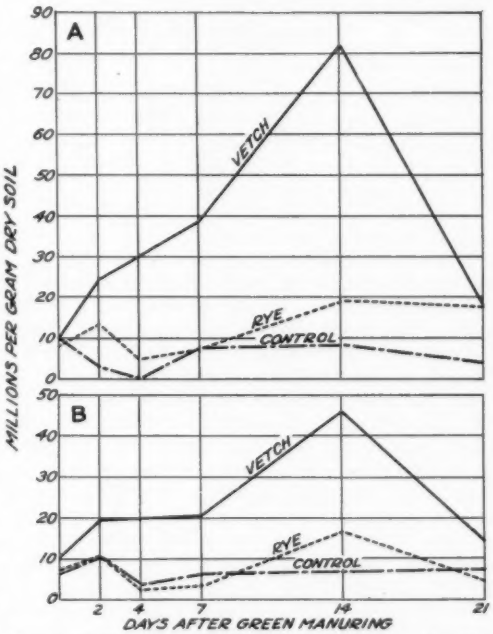


FIGURE 12.—Number of Gram-negative (A) and Gram-positive (B) bacteria in Keysport clay loam, as affected by rye and vetch green manures

The isolation of cultures was stopped with the 21-day sampling. It is not likely that any radical change would have been found at later samplings, for the total counts (fig. 7) continued to decrease and were on a level with the control at 56 days.

## EFFECT OF TREATMENTS ON ACTINOMYCETES

The number of actinomycetes present in the soil samples were determined as described above. The counts on the Collington and Leonardtown soils of the rye green-manuring series are given in Table 2.

TABLE 2.—*Millions of actinomycetes per gram of dry soil in two acid soils in the rye green-manure series when limed and unlimed*

| Days after green manuring | Collington fine sandy loam | | | | Leonardtown clay loam | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Rye | Limed rye | Control | Limed control | Rye | Limed rye |
| 0 | 2.6 | 3.0 | 3.2 | 4.0 | 2.4 | 2.6 | 3.0 | 2.9 |
| 2 | 2.7 | 3.3 | 4.4 | 3.5 | 3.4 | 4.2 | 3.0 | 3.0 |
| 4 | 3.4 | 4.3 | 3.4 | 4.5 | 3.4 | 3.0 | 3.2 | 2.6 |
| 7 | 2.9 | 3.1 | 2.4 | 2.4 | 3.4 | | 3.7 | 2.3 |
| 14 | 2.7 | 3.6 | 2.8 | 3.4 | 3.6 | 3.9 | 3.0 | 4.2 |
| 21 | 3.1 | 3.8 | 3.5 | 4.8 | 3.4 | 3.2 | 3.9 | 4.5 |
| 35 | 2.2 | 3.0 | 3.7 | 4.1 | 2.5 | 1.9 | 3.5 | 2.8 |
| 56 | 2.0 | 3.4 | 3.6 | 5.5 | 3.8 | 3.5 | 3.5 | 2.9 |
| 84 | 1.4 | 2.0 | 1.3 | 1.9 | 2.3 | 2.2 | 2.2 | 2.1 |

No significant increase in the number of actinomycetes occurred in the Leonardtown soil when limestone, rye green manure, or both were added. In the Collington soil there seems to have been a slight increase due to the treatments. On account of the great variations in the counts from one sampling to another, no importance should be attached to these small increases.

In the second experiment vetch was turned under in place of rye. The number of actinomycetes (Table 3) decreased slightly where vetch was added and rose slightly with limestone alone. This holds true for both soils. The decrease in the number of actinomycetes took place during the first seven days coincident with the highest counts of the bacteria. Millard and Taylor (*28*) found that a saprophytic actinomycete competed with a pathogenic form for the available food in green-manure experiments, and suggested that bacteria might also compete with weaker forms and inhibit their development. The bacteria may have inhibited the actinomycetes in the experiment here recorded. On the other hand, the actinomycete plates were heavily seeded with bacteria of the type producing an abundant growth. Therefore there may have been competition on the plates, with the result that a large number of the actinomycetes were either inhibited or so masked that the colonies could not be differentiated. The latter explanation seems the more plausible, especially since subsequent samplings showed, on the whole, the same number of actinomycetes as at the start.

TABLE 3.—*Millions of actinomycetes per gram of dry soil in two acid soils in the vetch green-manure series when limed and unlimed*

| Days after green manuring | Collington fine sandy loam | | | | Leonardtown clay loam | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Vetch | Limed vetch | Control | Limed control | Vetch | Limed vetch |
| 0 | 2.6 | 2.9 | 2.8 | 2.7 | | | | |
| 2 | 3.5 | 3.3 | 2.0 | 1.3 | 3.0 | 4.1 | 2.2 | 1.5 |
| 4 | 3.0 | 3.0 | 1.6 | 1.4 | 3.1 | 2.8 | 1.2 | 2.2 |
| 7 | | 2.4 | .8 | .7 | 1.9 | 1.8 | .9 | .6 |
| 14 | 1.5 | 3.5 | 2.2 | 2.3 | 1.9 | 2.2 | 1.9 | 2.0 |
| 21 | 2.2 | 2.4 | 1.9 | 2.5 | 2.1 | 2.8 | 2.4 | 1.9 |
| 35 | 1.5 | 1.8 | 2.1 | 2.4 | 2.1 | 2.6 | 1.7 | 1.8 |
| 56 | 2.1 | 2.0 | 2.8 | 2.7 | 2.6 | 2.7 | 2.6 | 2.8 |

As stated above, green rye and green vetch were again added to the same plots after several months of fallow under moist and rather hot conditions. Table 4 shows the counts of actinomycetes obtained. Here, again, there were lower counts in the fourth and seventh day samplings. The rye and vetch green manures on the acid plots of both soils seem to have inhibited the number of actinomycetes more than where limestone was also applied. This effect was not observed in the first application of the green manures. These results are rather surprising, for the reason that the total plate counts, especially of the limed green-manure plots of the Leonardtown soil, were at least three times those of the acid plots. In the Collington soil under the same conditions there was not quite so much difference between the limed and acid green-manure plots. With this soil, though, there was quite a difference in the plate counts of the acid vetch and acid rye plots. The actinomycetes in both cases, however, were the same. It seems, therefore, that it is not so much the number as it is the kind of bacteria which inhibits the development of the actinomycetes on the plates. The bacteria grew rapidly on the glycerin-nitrate agar and oftentimes spread over half the plate.

TABLE 4.—*Millions of actinomycetes per gram in two acid soils, limed and unlimed, after the second application of rye and vetch green manures*

| Days after green manuring | Collington fine sandy loam | | | | | | Leonardtown clay loam | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Rye | Limed rye | Vetch | Limed Vetch | Control | Limed control | Rye | Limed rye | Vetch | Limed Vetch |
| 0 | 2.4 | 2.3 | 1.4 | 1.7 | 1.7 | 2.7 | 2.4 | 3.7 | 1.9 | 2.4 | 2.2 | 2.2 |
| 2 | 3.1 | 3.0 | 2.2 | 3.0 | 2.7 | 2.3 | 2.5 | 2.8 | 2.8 | 3.1 | 2.9 | 3.8 |
| 4 | 2.4 | 2.2 | 1.4 | 2.2 | 1.4 | 1.9 | 2.3 | 1.7 | 1.1 | 2.0 | 1.8 | 2.0 |
| 7 | 2.7 | 2.9 | 1.5 | 2.9 | 1.6 | 3.4 | 3.2 | 5.0 | 2.0 | 2.0 | 1.4 | 2.4 |
| 14 | 2.8 | 2.5 | 2.2 | 2.9 | 2.8 | 4.8 | 3.3 | 3.5 | 2.9 | 2.6 | 3.3 | 3.2 |
| 21 | 2.8 | 3.1 | 2.0 | 3.1 | 3.0 | 4.0 | 3.3 | 4.0 | 3.0 | 3.1 | 3.1 | 3.3 |
| 28 | 3.6 | 4.1 | 3.3 | 3.8 | 2.4 | 3.9 | 4.2 | 4.2 | 3.6 | 3.4 | 4.1 | 3.2 |
| 42 | 2.1 | 1.9 | 1.4 | 1.5 | 1.4 | 1.7 | 1.4 | 1.7 | 1.6 | 2.2 | 1.5 | 2.2 |
| 63 | 1.6 | 2.8 | 2.1 | 1.4 | .6 | 1.8 | 2.7 | 1.9 | 2.7 | 3.1 | 2.0 | 3.0 |

The numbers of actinomycetes in the rye and vetch green-manure plots of Keysport clay loam are given in Table 5.

Although the numbers in this soil were about double those in the Collington and Leonardtown soils, no greater uniformity of counts was obtained; in fact, the fluctuations seem to be worse. Vetch caused

a decrease in the actinomycetes, probably by increasing the numbers of bacteria, some of which, as noted above, grew well on the medium. This is in harmony with the results of the other experiments. Rye, as noted above, failed to produce an increase in the bacteria in this soil. Therefore there was no crowding of the actinomycetes on the plates and the counts averaged the same as the control.

TABLE 5.—*Millions of actinomycetes per gram of dry soil in the field experiment on Keysport clay loam, rye and vetch green manures being used*

| Days after green manuring | Rye | Control | Vetch | Days after green manuring | Rye | Control | Vetch |
|---|---|---|---|---|---|---|---|
| 0 | 8.7 | 6.9 | 5.7 | 14 | 5.8 | 5.8 | 1.8 |
| 2 | 7.5 | 7.4 | 5.0 | 21 | 3.6 | 5.4 | 1.5 |
| 4 | 7.6 | 7.8 | 7.7 | 35 | 3.8 | 6.4 | 6.5 |
| 7 | 4.4 | 4.8 | 1.3 | 56 | 4.3 | 3.9 | 4.5 |

## EFFECT OF TREATMENTS ON FUNGI

For the determination of the number of fungi, plates were poured as described above and the colonies counted after three days. In Table 6 the counts on both the Collington and Leonardtown soils of the rye green-manure series are given. In both soils there was a slight increase in fungi on the second and fourth day samplings, especially on the unlimed Leonardtown soil. The increase was of short duration, for at 7 days the counts for the most part had returned to normal. At 14 days they seem to have decreased below the average. On both soils the highest counts of fungi were obtained on the unlimed soil, as might be expected. However, the increase in the fungi was really insignificant compared with the increase in the bacteria. The decomposition was, then, bacterial, and while the fungi did increase somewhat at first, they could not compete with the bacteria under these conditions.

TABLE 6.—*Thousands of fungi per gram of dry soil in two acid soils in the rye green-manure series, when limed and unlimed*

| Days after green manuring | Collington fine sandy loam | | | | Leonardtown clay loam | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Rye | Limed rye | Control | Limed control | Rye | Limed rye |
| 0 | 105 | 109 | 117 | 149 | 207 | 209 | 194 | 188 |
| 2 | 98 | 130 | 168 | 132 | 221 | 240 | 344 | 317 |
| 4 | 170 | 203 | 230 | 201 | 291 | 292 | 649 | 405 |
| 7 | 91 | 118 | 180 | 140 | 225 | 205 | 200 | 214 |
| 14 | 77 | 69 | 81 | 65 | 177 | 141 | 124 | 145 |
| 21 | 90 | 90 | 77 | 96 | 201 | 192 | 168 | 211 |
| 35 | 62 | 110 | 79 | 89 | 148 | 86 | 140 | 145 |
| 56 | 121 | 110 | 95 | 125 | 206 | 187 | 166 | 168 |
| 84 | 55 | 99 | 112 | 89 | 214 | 210 | 240 | 163 |

Vetch green manuring on these soils had even less effect than the rye upon the fungi, as shown by the results in Table 7. All the soils gave slightly lower fungi counts than when rye was turned under, but that is immaterial since the controls in this experiment were as uniform as in the former.

In this experiment, limestone seems to have increased the number of fungi somewhat in the Collington soil, whereas in the Leonardtown

soil it had the opposite effect. The effect in either case was slight if it be compared with that produced by the limestone upon the bacteria.

TABLE 7.—*Thousands of fungi per gram of dry soil in two acid soils in the vetch green-manure series when limed and unlimed*

| Days after green manuring | Collington fine sandy loam | | | | Leonardtown clay loam | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Vetch | Limed vetch | Control | Limed control | Vetch | Limed vetch |
| 0 | 74 | 100 | 108 | 85 | 169 | 115 | 170 | 122 |
| 2 | 102 | 126 | 96 | 102 | 188 | 191 | 117 | 222 |
| 4 | 98 | 98 | 100 | 99 | 149 | 154 | 75 | 98 |
| 7 | 75 | 104 | 103 | 102 | 157 | 182 | 192 | 185 |
| 14 | 85 | 97 | 91 | 106 | 199 | 167 | 140 | 168 |
| 21 | 99 | 89 | 122 | 117 | 131 | 144 | 196 | 164 |
| 35 | 100 | 87 | 77 | 87 | 142 | 140 | 198 | 128 |
| 49 | 97 | 99 | 93 | 102 | 184 | 180 | 198 | 178 |

The second application of rye and vetch green manures to those plots after a long fallow period had less effect upon the number of fungi than did the first application, as is shown in Table 8. It will be noted that the fungi had increased in the controls and also in the previously treated Leonardtown soil during the fallow period when no samples were taken. These increases in the number of fungi are not very large as compared with the increases observed in the total plate counts. The fact that the fungi failed to develop, especially in the acid green-manure plots, was very surprising and was one of the chief reasons for repeating the applications of green manures on these plots.

TABLE 8.—*Thousands of fungi per gram of dry soil in two acid soils, limed and unlimed, after the second application of rye and vetch green manures*

| Days after green manuring | Collington fine sandy loam | | | | | | Leonardtown clay loam | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Rye | Limed rye | Vetch | Limed vetch | Control | Limed control | Rye | Limed rye | Vetch | Limed vetch |
| 0 | 114 | 122 | 113 | 105 | 199 | 109 | 248 | 295 | 312 | 312 | 232 | 302 |
| 2 | 140 | 135 | 153 | 126 | 126 | 141 | 261 | 226 | 353 | 355 | 323 | 329 |
| 4 | 114 | 90 | 142 | 138 | 128 | 128 | 219 | 238 | 280 | 320 | 291 | 219 |
| 7 | 116 | 97 | 135 | 175 | 175 | 172 | 254 | 339 | 388 | 374 | 294 | 273 |
| 14 | 106 | 123 | 123 | 141 | 165 | 169 | 264 | 268 | 377 | 358 | 397 | 263 |
| 21 | 83 | 95 | 119 | 105 | 149 | 138 | 211 | 270 | 316 | 345 | 351 | 272 |
| 28 | 121 | 99 | 147 | 125 | 152 | 117 | 235 | 242 | 299 | 288 | 286 | 187 |
| 42 | 126 | 93 | 129 | 132 | 147 | 149 | 206 | 247 | 256 | 380 | 335 | 202 |
| 63 | 111 | 109 | 123 | 97 | 154 | 134 | 176 | 253 | 233 | 280 | 300 | 258 |

The effect of rye and vetch green manuring on the fungi in Keysport clay loam under field conditions is shown in Table 9.

TABLE 9.—*Thousands of fungi per gram of dry soil in the field experiment on Keysport clay loam, rye and vetch green manures being used*

| Days after green manuring | Rye | Control | Vetch | Days after green manuring | Rye | Control | Vetch |
|---|---|---|---|---|---|---|---|
| 0 | 138 | 159 | 150 | 14 | 178 | 190 | 200 |
| 2 | 137 | 152 | 180 | 21 | 147 | 153 | 192 |
| 4 | 163 | 179 | 126 | 35 | 152 | 178 | 196 |
| 7 | 165 | 196 | 180 | 56 | 165 | 151 | 198 |

Here, again, no increase in fungi is discernible. On the other hand, the number on the rye-treated plot is slightly smaller than on the control and vetch plots. This is just the reverse of what was expected. It had been thought that the less succulent condition of the rye green manure and the drier condition of the soil as compared with that in greenhouse experiments would stimulate the fungi.

No definite increase took place in the number of fungi at any time. These results are not in accord with those found by Waksman and Starkey (*39*). They reported that green manure increased both bacteria and fungi and that "the number of fungi increased more abundantly in the acid soils than in those more nearly neutral in reaction." Alfalfa meal was used as the green manure and mixed with soil in tumblers. Neller (*29*) decided that the fungi were the most active of the soil flora. He based his claim on the fact that pure cultures of these produced the same effects as a soil infusion when alfalfa meal was mixed with sandy soil in tumblers. The conditions of these experiments and those reported in this paper are so different it is no wonder that the results do not correlate. In studying the effects of green manure it would seem advisable to use the material in its natural condition and to incorporate it into the soil in such a manner that field conditions might be more nearly reproduced.

## EFFECT OF TREATMENTS ON THE ACCUMULATION OF NITRATES

In connection with the determination of the number of the bacteria, actinomycetes, and fungi, the amount of nitrogen as nitrates was determined on each soil sample. One hundred grams were weighed out and placed in a flask. To the Collington samples, 450 ml. of distilled water and 50 ml. of a 10 per cent suspension of aluminum cream, and to the Leonardtown samples, 400 ml. of distilled water and 100 ml. of the cream were added. The suspensions were shaken intermittently for an hour and filtered. Two 100 ml. aliquots were evaporated on the steam bath and the nitrates determined by the phenoldisulphonic-acid method. Correction for moisture was made and the results reported as parts per million. If the soil contained more than 50 parts per million of nitrate nitrogen, small aliquots were taken.

The nitrates in the first rye green-manure experiment on Collington fine sandy loam are shown in the lower part of Figure 1. There was a slow increase in nitrate nitrogen in the control plot, as might be expected of field soil which was brought into the greenhouse and kept fallow under optimum conditions.

In the limed soil the nitrates were somewhat higher throughout the experiment, indicating only a slight effect of limestone on the nitrification of the soil nitrogen.

In the rye green-manure plots (fig. 1), with or without limestone, there was a large increase in nitrates within 7 days. While there was somewhat of a drop at 21 days, the nitrates at 35 days had increased on an average to more than 60 parts per million and remained high thereafter. It is interesting to note that although the pH value of the original soil was 5.2, limestone apparently made no difference in the nitrification of the nitrogen of the rye green manure.

Analogous results were obtained on Leonardtown clay loam with rye green manure. (Fig. 2.) The drop in nitrates at the seventh-day sampling of the rye-limestone plot is inexplicable. Since the other data on the limed and acid rye plots are consistent, it may be assumed that this seventh-day determination was wrong. After 35 days the nitrification of the rye green-manure nitrogen was slowed up in the acid soil, whereas in the limed the nitrates rose to over 200 parts per million in 56 days. However, at 119 days the acid plot was nearly as high as the limed.

Vetch green manure on the Collington soil, either limed or unlimed (fig. 3), gave results similar to the rye, except that nitrification was slower. The nitrates in all the plots were practically the same up to the 21-day sampling. Then the soils receiving the application of vetch green manure definitely contained more nitrate nitrogen. This condition persisted to the end of the experiment at 56 days.

On the Leonardtown soil (fig. 4), vetch green manure gave erratic results. It will be remembered that the total plate counts on these plots were not in accord with the other experiments, especially in regard to the behavior of the acid vetch plot. The nitrates in the control soil increased slowly and those in the limed soil more rapidly. The vetch-limestone plot showed the largest increase, reaching 100 parts per million at 56 days. The limed control soil at this period contained about 80 parts per million of nitric nitrogen.

When the second applications of rye and vetch were made on the respective plots of the Collington soil six months after the first, the nitric nitrogen had increased considerably in all plots. The limed green-manure plots had the largest amount, the acid rye and vetch the next largest, and the limed and control plots the smallest. (Fig. 5.) In two days after green manuring there was a decrease in all cases. This was partly due, perhaps, to stirring the soil, since the two controls also showed the decrease, and partly to the tremendous growth of bacteria which took place within 14 days, especially in the limed green-manure plots. Without limestone, nitrification was delayed, nitrates not accumulating in the rye or vetch plots until the forty-second and sixty-third day samplings.

The results of the second applications of rye and vetch on the Leonardtown plots gave similar though more striking results. (Fig. 6.) The nitrate content of all the treated plots was between 190 and 245 parts per million when the green manure was turned under. Within 7 days it had decreased markedly, only to rise again at 14 days. After another slight decrease, nitrates rose rapidly at the last two determinations and in most cases attained the same height as at the beginning of this experiment.

The effect of rye and vetch green manures on the nitrates in the field soil is shown in Figure 7. Rye green manure failed in this instance to increase the nitric nitrogen at any time during the experiment. The vetch treated plot, on the other hand, showed an increase beginning at 21 days and amounting to over 50 parts per million in 56 days.

The rapid accumulation of nitrates under optimum conditions after green manuring has been observed by many investigators. Joshi (*25*) found that Crotolaria, Sesbania, and cowpeas nitrified rapidly under

laboratory conditions. In 1 week nitrates were 45 to 70 parts per million; in 2 weeks, 85 to 120; and in 3 weeks, 110 to 145. Similar results were obtained by Whiting and Schoonover (*42*) when green clover tops were added to soil in gallon jars kept in the greenhouse. These and the results obtained by others are in accord with the data presented in this paper.

Nitrification took place in the acid plots and to practically the same extent as in the neutral plots. Hall, Miller, and Gimingham (*22*) reported that nitrification went on slowly in the very acid plots. They considered it as being localized around particles of limestone where the reaction was not so acid. Abbott, Conner, and Smalley (*1*) found that nitrification progressed as rapidly when 4,000 pounds $CaCO_3$ were needed as when it was added.

Brown and MacIntire (*9*), Temple (*37*), White (*40*), Fred and Graul (*20*), Stephenson (*36*), and others have reported active nitrification in acid soils. Olson (*31*) states that nitrification can take place at a pH of 3.7. In the present work the pH value of the Leonardtown soil toward the end of the first vetch green-manuring experiment was 4.2. The nitrate content at that time was only slightly lower than where limestone had also been added, showing that nitrification had gone on at this low pH value.

As a rule, nitrates were low when the bacteria were high. There are exceptions, notably in the first experiments. No explanation has been found for this with the data at hand.

The decrease in nitrates immediately after the second application of the green manures is very striking. After nine weeks they had accumulated again to the former high levels, but no higher. The bacteria at this time were low. Additional analyses for bacterial numbers and nitrates were made a few months after this experiment. The bacteria had increased somewhat and the nitrate had decreased. It is still a problem as to what became of the nitrogen added in the second green manure. At least it did not increase the amount of nitrates already in the soil.

## EFFECT OF TREATMENTS ON SOIL ACIDITY

The hydrogen-ion concentration of each sample was determined to see if any change in the reaction of the soil took place during the process of decomposition. The quinhydrone electrode was used and the procedure was as follows: 5 to 10 gm. from each sample were placed in a test tube and a small quantity of quinhydrone added. A sufficient amount of distilled water was added to make the proportion of soil to water 1 : 1 to 1 : 2. A standard solution consisting of 0.09 N KCl in 0.01 N HCl and having a pH of 2.04 at 18° C. was poured into another tube. The standard solution was then connected with the soil suspension by a saturated KCl agar bridge. Clean platinum electrodes were used. The difference in electromotive force was determined on a potentiometer and correction made for temperature.

The results when rye was used as the green manure are given in Table 10.

TABLE 10.—*pH values of samples from two acid soils, limed and unlimed, in the rye green-manure series*

| Days after green manuring | Collington fine sandy loam | | | | Leonardtown clay loam | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Rye | Limed rye | Control | Limed control | Rye | Limed rye |
| 0 | 5.1 | 6.6 | 5.2 | 6.5 | 4.4 | 6.5 | 4.6 | 7.3 |
| 2 | 5.2 | 7.0 | 5.2 | 6.9 | 4.4 | 7.1 | 4.6 | 7.5 |
| 4 | 5.3 | 6.3 | 5.3 | 6.9 | 4.6 | 6.8 | 4.6 | 7.1 |
| 7 | 4.7 | 6.6 | 5.0 | 6.8 | 4.6 | 7.2 | 4.6 | 7.4 |
| 14 | 4.8 | 6.4 | 4.8 | 6.5 | 4.5 | 6.8 | 4.4 | 7.1 |
| 21 | 4.7 | 6.7 | 4.6 | 6.6 | 4.4 | 6.7 | 4.2 | 7.3 |
| 35 | 5.1 | 6.5 | 4.8 | 6.6 | 4.5 | 7.0 | 4.2 | 7.2 |
| 56 | 5.3 | 6.9 | 4.8 | 6.8 | 5.1 | 7.0 | 4.4 | 7.0 |
| 84 | 4.7 | 6.7 | 4.5 | 6.8 | 4.2 | 6.9 | 4.0 | 7.2 |
| 119 | 4.8 | 6.8 | 4.7 | 6.6 | 4.2 | 6.7 | 4.1 | 7.2 |

A definite change in the hydrogen-ion concentration of the soil took place during the experiment. The unlimed rye green-manure plots of both soils became more acid toward the end. This was probably due to the production of nitric nitrogen which, as was shown above, took place in these acid soils. Where limestone was added, the acidity, of course, was taken care of by the excess of limestone added. In comparing the limed control with the limed rye plot of the Leonardtown soil, it is seen that the pH value of the latter is higher throughout the experiment. Six months later the pH value was the same in both, being slightly above 7. As the same amount of limestone was added to both plots, the only explanation seems to be that the limestone was not evenly distributed in this particular case. The determinations as a whole leave much to be desired in the way of uniformity.

The effects produced on the pH values by vetch green manure were similar to those produced by the rye. (Table 11.) Although the experiment lasted only 56 days, there was a definite production of acidity by that time where the limestone was not added. However, in the early stages of decomposition in the Collington soil there seemed to be less acidity; that is, a production of alkali. More detailed experiments along this line are being carried on.

TABLE 11.—*pH values of samples from two acid soils, limed and unlimed, in the vetch green-manure series*

| Days after green manuring | Collington fine sandy loam | | | | Leonardtown clay loam | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | Limed control | Vetch | Limed vetch | Control | Limed control | Vetch | Limed vetch |
| 0 | 4.9 | 6.5 | 5.0 | 6.4 | 4.6 | 7.4 | 4.6 | 7.3 |
| 2 | 4.8 | 6.4 | 5.4 | 6.5 | 4.6 | 7.0 | 4.7 | 6.6 |
| 4 | 5.0 | 7.0 | 5.4 | 7.1 | 4.5 | 7.4 | 4.6 | 7.3 |
| 7 | 5.0 | 6.7 | 5.2 | 7.2 | 4.6 | 7.3 | 4.7 | 7.2 |
| 14 | 4.8 | 6.9 | 4.9 | 7.0 | 4.5 | 7.2 | 4.5 | 7.3 |
| 21 | 4.9 | 6.8 | 4.9 | 6.6 | 4.4 | 7.3 | 4.5 | 7.2 |
| 35 | 4.8 | 6.8 | 4.6 | 6.8 | 4.3 | 7.3 | 4.2 | 7.1 |
| 56 | 4.7 | 6.7 | 4.4 | 6.6 | 4.3 | 7.0 | 4.2 | 7.2 |

The second application of rye and vetch green manures made some six months after the first application gave similar results. Again it was noted that when vetch green manure without limestone was used less acidity was present during the first seven days, especially in the

Collington soil. The Leonardtown soil, being heavier, contains more buffer, so that the small amounts of acid and alkali produced failed to show in the determinations as made. The table giving these data on the second application of the green manures has been omitted, since it contains only confirmatory information.

The effect of green manuring upon Keysport clay loam under field conditions is shown in Table 12. If the rye and vetch green-manured plots are compared with the control for each sampling, no change in pH values is observed, except in one case. At seven days, vetch seemed to have produced some alkali; but since all the determinations show considerable fluctuations from one sampling to another, it is unsafe to place any dependence upon this determination. The Keysport soil is heavily buffered and would be expected to absorb the small amounts of acid and alkali which might be produced in the decomposition of the green manure and the mineralization of its nitrogen.

TABLE 12.—*pH values of Keysport clay loam as affected by green manuring in the field experiment*

| Days after green manuring | Rye | Control | Vetch | Days after green manuring | Rye | Control | Vetch |
|---|---|---|---|---|---|---|---|
| 0 | 5.5 | 5.5 | 5.6 | 14 | 5.8 | 5.8 | 5.9 |
| 2 | 5.0 | 5.1 | 5.4 | 21 | 5.8 | 5.7 | 5.8 |
| 4 | 5.6 | 5.5 | 5.7 | 35 | 5.6 | 5.6 | 5.6 |
| 7 | 5.6 | 5.6 | 6.1 | 56 | 5.3 | 5.5 | 5.5 |

Nitrification renders the medium wherein it takes place more acid. In these experiments the tendency was noted for the control and unlimed plots to become slightly more acid with the increase in the amount of nitrates. White (*41*) stated that the increase in soil acidity in his experiments could not be explained as due to nitrification. While nitrification was a factor, it was by no means the chief one. Howard (*24*) found that rye green manure produced an acid reaction equivalent to 300 to 450 pounds CaO per acre and vetch only half this amount.

Even in a soil containing more than 3 per cent $CaCO_3$, Sackett and his associates (*34*) were able to detect an increase in the hydrogen-ion concentration due to the application of green manure. On the other hand, Ames and Schollenberger (*2*) found that both dry and green materials reduced the lime requirements at the end of three months. Other work might be referred to in which it is stated that green manure produces a more acid reaction or tends to neutralize an acid soil. The data are therefore rather conflicting. This is no doubt due to the varying conditions of the experiments. In the present work an increase in acidity is correlated with an increase in nitrate content. What effect the absorption of these nitrates by growing plants would have upon the soil acidity can not be stated. However, there is no reason to suppose that this acidity would be permanent.

## SUMMARY

The effect of the addition of rye or vetch green manures to Collington fine sandy loam and to Leonardtown clay loam under optimum conditions of temperature and moisture and with and without limestone may be summarized as follows:

Green manure on both the acid and neutral plots increased the number of soil microorganisms as determined on soil-extract agar.

The greatest number usually occurred in four days and was followed by a rapid decline coincident with the disappearance of the leafy portion of the green manure.

In the acid green-manured soil the total plate counts remained low after the decline. In the soil neutralized with limestone and treated with green manure a secondary increase took place and the number remained high.

Limestone caused an increase in the total plate count five weeks after application.

A second application of green manures to the same soil six months after the first showed the same great increase in microorganisms during the first few days. This was followed by a reduction in numbers which continued to the end of the period of observation. No secondary rise took place as was noted in the first experiments.

The increase in the soil microorganisms was due to the growth of bacteria. Both the Gram-negative and Gram-positive bacteria increased greatly during the active decomposition of the green manure.

The secondary rise in bacterial numbers due to the effect of limestone was caused by an increase in the Gram-negative organisms.

The number of fungi in the acid or neutralized soils was not influenced to any great extent by the addition of green manures.

The number of actinomycetes was lower during the greatest development of the bacteria. This was due to overcrowding of the plates by certain rapidly growing bacteria. Otherwise no effect of the green manures was apparent.

Green manures stimulated the accumulation of nitrates in both the acid and neutralized soils, the amount of nitrates being slightly lower in the acid soil. Although there were many irregularities, nitrates tended to be high when the bacteria were low, and vice versa.

There was a tendency for the control soils to become slightly more acid as the nitrates accumulated. With the addition of green manure and a greater accumulation of nitrates, there was a definite increase in acidity on the sandy soil where no limestone was added. This was not so noticeable in the clay soil.

In the early stages of decomposition small but significant amounts of alkali were produced in the sandy soil which reduced the acidity. Practically no effect was observed on the clay soil.

Vetch green manure under field conditions decomposed more slowly than under greenhouse conditions, but the effects were similar to those obtained in the greenhouse. Rye green manure under these conditions produced practically no change in the number of soil microorganisms, in the amount of nitrate, or in the hydrogen-ion concentration of the soil.

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# NEODIPLOGASTER PINICOLA, N. SP., A NEMA ASSOCIATED WITH THE WHITE-PINE WEEVIL IN TERMINAL SHOOTS OF THE WHITE PINE[1]

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## INTRODUCTION

Fuchs (*4*)[2] in 1915 first called attention to the fact that the moist frass of the mines produced by *Ips typographus* and *Hylobius abietis* is inhabited by a specific nemic fauna, the members of which are in more or less close symbiotic relationship with the beetles. He observed that some of the nemas were carried about by the beetles, either under the wing covers or in some other way. In some instances the relationship between nemas and beetles seemed to be more or less that of parasite and host. Fuchs also mentioned the fact that different genera and species of beetles have different nemas as their associates. Similar observations were published more recently by Jazentkovsky (*5*).

Previous to the present paper, so far as the writer is aware, no case of the association of a nema with the white-pine beetle (*Pissodes strobi*) has been recorded.

In June, 1928, R. L. Taylor, of the Bussey Institution for Research in Applied Biology, Harvard University, sent the writer terminal shoots of the white pine (*Pinus strobus*) affected by *Pissodes strobi* and harboring numerous nemas in the mines. Doctor Taylor at that time suggested that probably the same nemas also occur under the wing covers of the beetles. Further material for study was then received from Maine through the courtesy of E. G. Arzberger. As far as the writer could see, the mines of *P. strobi* harbor only one species of nema, which, however, is new and belongs to the genus Neodiplogaster Cobb (*2*).

## RELATIONSHIPS OF GENUS NEODIPLOGASTER

Neodiplogaster is closely related to the genus Diplogasteroides of De Man (*6*) and also to the genus Rhabditolaimus established by Fuchs (*4*) for forms that he found in the mines of the bark beetles of Europe. Only one species of Neodiplogaster is known at present—*N. tropica* which Cobb (*2*) found in coco pods from Guatemala. The status of the genus at that time was still somewhat doubted by its author. After making a study of the present form, the writer considers Neodiplogaster a good genus. Its relationship to the other genera of the Diplogasteridae is best shown by a summary of the diagnostic characters of the various genera:

DIPLOGASTEROIDES (De Man, *6*).—Diplogasteridae with a cylindrical undivided pharynx, at the base of which is a small dorsal tooth; male tail with numerous papillae but no bursal structures.

[1] Received for publication Mar. 18, 1930; issued July, 1930.

[2] Reference is made by number (italic) to Literature Cited, p. 129.

RHABDITOLAIMUS (Fuchs, *4*).—Diplogasteridae similar to Diplogasteroides, but with the pharynx unarmed; male tail with small bursal membranes.

NEODIPLOGASTER (Cobb, *2*).—Diplogasteridae with the pharynx divided into a short but wide anterior section and a longer cylindrical posterior section; on the bottom of the anterior part a single well-developed dorsal tooth; on the bottom of the posterior part two subdorsal cuticularized oval pieces. Male tail with numerous papillae partly connected by more or less developed bursal structures.

DEMANIELLA (Steiner, *10*).—Diplogasteridae with cylindrical undivided pharynx, at the bottom of which is a large dorsal tooth; anterior esophageal bulb elongated; oral opening with conical elevation and surrounded by a number of semicircular cuticular thickenings.

ODONTOPHARYNX (De Man, *7*).—Diplogasteridae with irregularly shaped, wide, deep, armed pharynx; anterior part of esophagus without distinct bulb but its lumen throughout its entire length with highly cuticularized walls, and the esophageal tissue with strong radial muscles. Male tail with numerous papillae but no bursal structures.

## CLASSIFICATION OF THE DIPLOGASTERIDAE

As against Baylis and Daubney (*1, p. 41–42, 109–110*), the writer holds the opinion that Diplogaster, Rhabditolaimus, Odontopharynx, Demaniella, Neodiplogaster, and Diplogasteroides are good genera, each including perhaps a number of species. They form a natural family (the Diplogasteridae), in which the structure of the posterior part of the esophagus is the chief distinguishing characteristic. Rhabditolaimus and Diplogasteroides, which are among those genera of the Diplogasteridae that show a definite relationship to the genus Rhabditis (family Rhabditidae), have the typical diplogastroid esophagus—that is to say, the terminal bulb is without radial muscle fibers and has no valvular apparatus.

The subgenus Rhabditella, of the genus Rhabditis, may be considered a connecting link between Rhabditolaimus and Diplogasteroides on the one hand and Rhabditis on the other, for in Rhabditella the male tail end very closely resembles that of either of the two diplogastroid genera. Rhabditella is nevertheless clearly distinguished from both of these Diplogasteridae by the shape of the posterior part of the esophagus, the terminal bulb of which has a definite valvular apparatus.

The Diplogasteridae possess additional distinguishing characters in the male copulatory apparatus with its spicula and gubernaculum and in the arrangement and shape of the papillae. The papillae, however, are sometimes supporting membranes of bursal character, thus clearly showing a relationship to Rhabditella. Moreover, in some forms (Diplogasteroides, Rhabditolaimus, etc.) the pharynx exhibits a close relationship to the typical Rhabditis pharynx.

The present classification of the Diplogasteridae is not considered as final. Cobb (*3*) has already called attention to the fact that the forms at present included in the genus Diplogaster represent various groups with distinct characters. The writer is convinced that in the near future these groups will be definitely separated into different genera.

## HABITS OF THE WHITE-PINE WEEVIL

The white-pine weevil, *Pissodes strobi*, occurs wherever the white pine grows, but is economically important in only a part of the range of the white pine. Its principal damage is produced on this plant, although a few others like *Pinus rigida* (pitch pine), *Picea rubra* (red spruce), *P. abies* L. (Norway spruce), *Abies balsamea* L. (balsam fir), and *Tsuga canadensis* L. (hemlock) may also be attacked. The beetle causes in the pine the abnormal development that is called "cabbage" or "pasture" pine. It attacks the terminal shoots of the host plants, killing them off; new leading shoots may then be developed and in turn killed, the result being that often a large number of new terminal shoots are formed which give the host plant the forked and crooked aspect previously mentioned.

The white-pine weevil lays its eggs in small holes that it makes in the topmost shoot of the previous year's growth. A number of eggs are usually deposited in one hole, and the newly hatched larvae gnaw small mines in the tissue below the bark. Usually only some of the eggs develop, and the mortality of the larvae is very high, presumably from starvation or parasitism. The mature larvae bore into the wood parenchyma and pupate. The adults emerge in the latter part of August or still later and are supposed to hibernate in the ground (Peirson, *9*).

## NEODIPLOGASTER PINICOLA N. SP.

### ECONOMIC IMPORTANCE

The life cycle of the nema observed by the writer as a parasite of the white-pine weevil is not yet known, but the fact is established that the adult beetle carries specimens of the nema under its wing covers, and only by this fact can the presence of the nema in all the mines examined be understood. It is considered probable that when the beetle deposits its eggs some of the nemas slip out from under the wing covers and remain with the eggs in the new mine.

Here, perhaps, the nema comes in as a control factor of economic value by causing the high mortality in the eggs and young beetle larvae previously mentioned, although this has not yet been verified by observation or experiment. Diplogaster species, however, have been observed feeding on grasshopper eggs (Merrill and Ford, *8, p. 124*). This fact and observations on other Diplogasteridae suggest the possibility that the present form may feed on the eggs of the beetles.

### TECHNICAL DESCRIPTION

**Neodiplogaster pinicola, n. sp.**

*Neodiplogaster pinicola* (fig. 1) is slender and of small size, not quite 1 mm. in length. The tail end of the larva and of the female is long-conical and sharply pointed. That of the male is much shorter but also conical and pointed. The cuticle is annulated, but with high magnification the annulation is resolved into series of transverse and longitudinal dots. These dots are more distinct on the lateral surface, especially at the tail end. Figure 1, F, gives a view of the arrangement of these dots. A wide undotted space always separates a double longitudinal series of dots except along the lateral line where four such series of dots are close together. (Fig. 1, F.) If, however, these series of dots are analyzed, it can be seen at once that groups of four dots alternate with groups of only two, which are placed in a transverse direction. This arrangement gives the surface a very specific character. The head end is not set off; its shape is convex-conoid. A

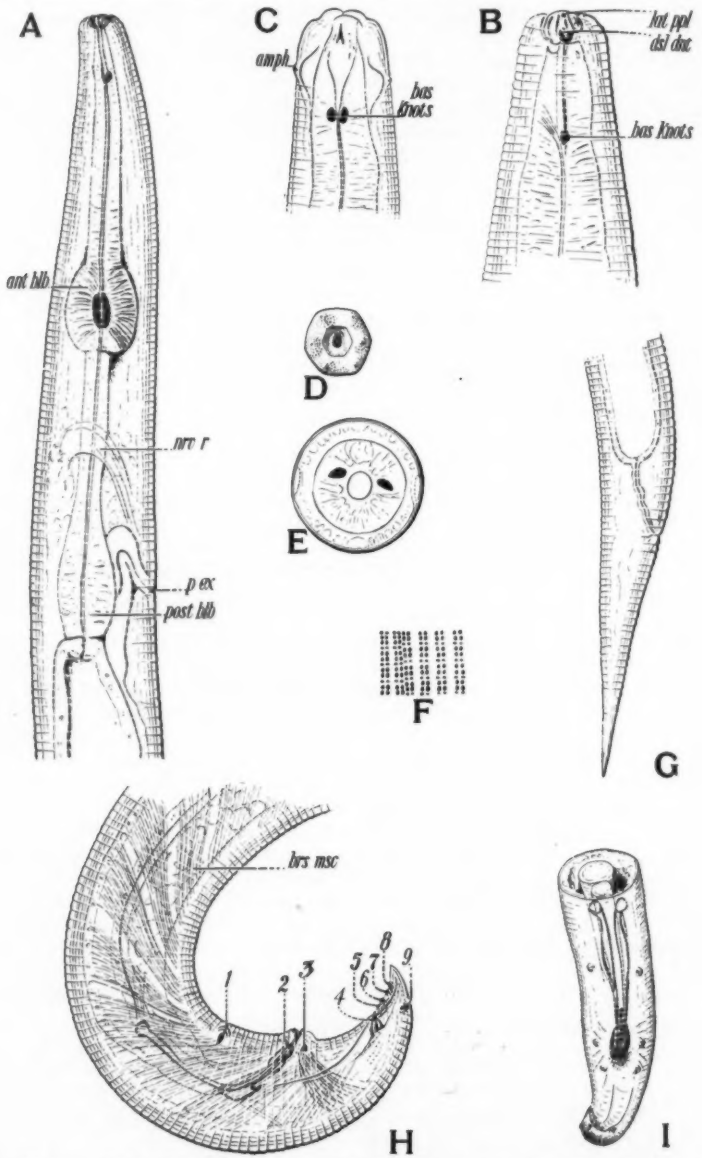


FIGURE 1.—*Neodiplogaster pinicola*, n. sp. A, Anterior end; *ant blb*, anterior esophageal bulb; *nrv r*, nerve ring; *p ex*, porus excretorius; *post blb*, posterior esophageal bulb. × about 700. B, Lateral view of head end; *bas knots*, cuticularized dorso-sublateral basal knots of pharynx; *dsl dnt*, dorsal onchium; *lat ppl*, lateral papilla. × about 1,433. C, Medial view of head end; *bas knots*, cuticularized dorso-sublateral basal knots of pharynx; *amph*, amphid. × about 1,433. D, Front view of head. × about 1,433. E, Cross section in region of basal knots of pharynx. × about 1,433. F, Arrangement of dots on cuticle. G, Tail end of female. × about 700. H, Lateral view of male tail end; *brs msc*, bursal muscles; 1–9, male copulatory papillae. × about 700. I, Ventral view of male tail end. × about 700

circle of six papillae is present. The amphids are very inconspicuous and open behind the middle of the pharynx. The latter is separated into two parts, a wide but shorter anterior one, which is striated longitudinally, and a narrow but longer posterior one. At the base of the anterior portion of the pharynx a single dorsal tooth is seen; it is quite conspicuous and curves forward. The second part of the pharynx is narrower but much longer and of somewhat cylindrical shape. Its shape in cross section was not made out. Two ellipsoidal cuticularized structures are seen at the base of this pharyngeal section, much as in *Neodiplogaster tropica;* they seem not to protrude into the pharyngeal cavity but to be located in the surrounding tissue in a somewhat dorso-sublateral position. (Fig. 1, A, B, C, E.) Their significance is not clear. The esophagus is of typical diplogastroid shape. As can be seen in Figure 1, A, the anterior esophageal bulb is very well developed. The male sexual apparatus has a single testis, outstretched forward. The bursal muscles are strongly developed, eight pairs being located in front of the anus and two behind it. The spicula are arcuate, exceedingly slender; the proximal ends are cephalated, the distal ends sharply pointed. The gubernaculum completely surrounds the spicula; it is of very complicated structure, as may be seen in Figure 1, H, I. A series of about nine ventro-submedial papillae was seen on each side of the male tail. The arrangement is very specific and furnishes an outstanding differentiating feature between *Neodiplogaster tropica* and the present species. The first papilla is near the middle of the spicula, the second is just in front of the anus, the third just behind it. These three papillae are not of identical size, the first one being larger than the other two. A fourth papilla is located ventro-submedially just behind the middle of the tail; it seems to support a small membranous structure, a kind of miniature bursa. A series of four much smaller papillae is seen in front of the tail end, close to the ventro-medial line. The ninth papilla, however, has a dorso-submedial position a short distance in front of the tail end. The female reproduction apparatus is amphidelph and the ovaries are reflexed. The vulva opens just behind the middle of the body.

MEASUREMENTS:

| | | | | | | |
|---|---|---|---|---|---|---|
| ♀ | 2.6<br>1.5 | 10.1<br>2.1 | 14.4<br>2.7 | 20 (55.5) 22<br>3.8 | 93.8<br>2.6 | 0.907 mm. (0.834 mm.–0.980 mm.) |
| ♂ | 2.8<br>1.5 | 12.<br>3.4 | 18.5<br>3.5 | 62.5<br>—M<br>4.7 | 92.2<br>3.4 | 0.687 mm. (0.66 mm.–0.713 mm.) |

DIAGNOSIS OF THE NEW SPECIES.—Neodiplogaster differing from *Neodiplogaster tropica* mainly in the different arrangement of the male copulatory papillae, in the much more reduced bursa, and in the presence of a dorso-submedial papilla close to the end of the male tail. Wall of anterior portion of pharynx with longitudinal striae.

## SUMMARY

A new nema, *Neodiplogaster pinicola*, n. sp., is described, and the affinities of this and other genera of the Diplogasteridae are reviewed. The present form was found living in the moist frass of mines produced by *Pissodes strobi*, the white-pine weevil, in terminal shoots of the white pine (*Pinus strobus*). It is suggested that this nema may be of economic importance as an enemy of its host, on the eggs and larvae of which it probably preys.

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# A METHOD FOR DETERMINING THE QUANTITY OF OIL RETAINED BY CITRUS FOLIAGE AFTER SPRAYING[1]

By L. L. ENGLISH

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## INTRODUCTION

In order to determine the effect of oil emulsions on Satsuma orange trees and on the insects which infest their foliage, it is desirable to know how much oil remains on the foliage after spraying. Laboratory and field experiments were therefore conducted in an effort to develop a method for determining the amount of oil retained by the foliage. The method herein reported was finally adopted.

## MATERIAL AND EQUIPMENT

To avoid the laborious and time-consuming operation of measuring leaves with a planimeter, the method of cutting disks from the leaves, suggested by Ginsburg,[2] was used. A 10 sq. cm. disk proved to be a convenient size. Including both sides of the leaf, 50 such disks make a sample having a total area of 1,000 sq. cm. Even though the determinations represent the amount of oil both on the leaf and in the leaf, it was thought advisable to base the calculations on the area sprayed.

The graduations on the Babcock bottles used were calibrated to read in cubic centimeters rather than in percentage by running a drop of mercury into the capillary, measuring its length, and then weighing it. A uniform lot was obtained that calibrated 0.002 c. c. for each division of the tube. The regular heating unit in an oven was replaced with a 100-watt electric-light bulb to make it safe for concentrating ether extracts. A standard type of centrifuge equipped with an electric heater was used.

## PROCEDURE

The emulsion was applied to duplicate trees with a power sprayer. The concentration of the emulsion at the nozzle was checked by a slight modification of the Lichtenberg[3] method, which has been found more satisfactory than the method in which only sulphuric acid is used. The day after application, or as soon as the spray dried, 50 leaves were clipped from various parts of each tree. A 10 sq. cm. disk was punched from each leaf. The 50 disks were placed in a 6-ounce wide-mouthed bottle, 50 c. c. of ether were added, and the

[1] Received for publication Jan. 29, 1930; issued July, 1930. This paper is based on cooperative investigation between the Agricultural Experiment Station of the Alabama Polytechnic Institute and the Alabama State Department of Agriculture.

[2] GINSBURG, J. M. AN APPARATUS FOR OBTAINING MEASURED AREAS OF SPRAYED FOLIAGE FOR CHEMICAL ANALYSES. Jour. Agr. Research 36: 1007-1009, illus. 1928.

[3] LICHTENBERG, H. F. A RAPID METHOD FOR THE DETERMINATION OF FAT IN ICE CREAM. Indus. and Engin. Chem, 5: 786. 1913. (Letter to the editor.) This is a method for the determination of the amount of fat in ice cream. The procedure calls for 9 gm. of the sample, 20 c. c. of glacial acetic acid, and 10 c. c. of sulphuric acid. This was modified to the following: 10 c. c. of oil emulsion, 15 c. c. of glacial acetic acid, and 10 c. c. of concentrated sulphuric acid, chemically pure. The mixture is shaken after the addition of each acid. It is then centrifuged, hot water added, recentrifuged, and read. The determination is carried out in Babcock 8 per cent milk bottles or skim-milk bottles, depending upon the concentration of the emulsion.

bottle was closed with a cork stopper and shaken by hand for one minute. The extract was filtered through a fast filter paper into a 125 c. c. Erlenmeyer flask. Another 50 c. c. portion of ether was added and the extraction repeated. The filter paper was washed with 10 c. c. of ether. The extract was concentrated to 20–25 c. c. in the open air and transferred to a Babcock skim-milk bottle. Five cubic centimeters of approximately 0.5 N sulphuric acid were added as a floating medium. (The loss of oil due to sulphonation is negligible, even if the unsulphonated residue of the oil is low.) The remainder of the ether was driven off in an electric oven at 50° C. The temperature was gradually raised to 80° to insure removal of all traces of ether. Enough hot, dilute sulphuric acid (0.5 N) was added to almost fill the bottle. It was heated to 80°–85°, centrifuged at approximately 2,000 revolutions per minute in a heated centrifuge, reheated until the oil flowed freely, and more acid was carefully added in successive portions with reheating and recentrifuging to bring the oil gradually up into the capillary tube, where it was read at room temperature with a pair of dividers.

## EXPERIMENTAL RESULTS

Table 1 shows the results from samples of leaves collected from trees sprayed with an oil emulsion at a volume concentration of 3 per cent. The determinations from two trees agreed very closely, and the amount of oil extracted from the disks checked satisfactorily with the amount from the whole leaves. The determination from sample C indicates that all the oil was obtained by two successive extractions with 50 c. c. of ether.

TABLE 1.—*Amount of oil extracted from disks and whole leaves collected from duplicate trees sprayed with an oil emulsion of 3 per cent concentration by volume*

| Sample | Method of extraction | Millionths of a cubic centimeter of oil per square centimeter on leaves from— | |
|---|---|---|---|
| | | Sprayed trees | Check trees |
| A | Fifty 10 sq. cm. disks given 2 successive extractions | 64.0 | 22.0 |
| | | 62.0 | 20.0 |
| B | Twenty whole leaves extracted same as A | 65.3 | 20.3 |
| | | 67.3 | 21.5 |
| C | Disks from A reextracted | 2.0 | 2.0 |
| | | 2.0 | 2.0 |

Table 2 gives examples of the results which may be expected from the use of this method. In this table, data are shown from five trees in each of two separate blocks sprayed with different emulsions. The variations are probably due to differences in individual trees and imperfections in spraying rather than to inaccuracies in the method itself.

TABLE 2.—*Amount of oil obtained from five random samples collected from two blocks of sprayed trees*

| Sample No. | Millionths of a cubic centimeter of oil per square centimeter of leaf in— | | | Sample No. | Millionths of a cubic centimeter of oil per square centimeter of leaf in— | | |
|---|---|---|---|---|---|---|---|
| | Block 1 | Block 2 | Check | | Block 1 | Block 2 | Check |
| 1 | 62 | 60 | 24 | 4 | 62 | 68 | 24 |
| 2 | 62 | 56 | 24 | 5 | 52 | 68 | (a) |
| 3 | 58 | 64 | 24 | | | | |

[a] Fifth sample not collected because of rain.

Allowing disks to stand in ether overnight, or extracting in a Soxhlet apparatus for six hours, did not increase the net amount of oil. These more drastic extractions merely increased the gross determination on both treated and check trees. This is undesirable in making determinations from foliage on which there is only a small amount of oil. The high melting point of the residue from check trees also makes the determination more difficult. There is no advantage in extracting with a mixture of ether and benzin in place of ether. On the contrary, it is often difficult to remove the last trace of benzin, even though it has previously been redistilled at a temperature not exceeding 60° C.

## SUMMARY

A practical method is reported for determining the amount of oil retained by citrus foliage after spraying with oil emulsions. The procedure consists of the extraction of oil with ether from measured areas of foliage and its subsequent recovery and measurement in Babcock skim-milk bottles.

# THE VALUE OF POTASSIUM IODIDE AS A SUPPLEMENT TO THE RATION OF GROWING CHICKS[1]

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## INTRODUCTION

That birds as well as mammals respond to iodine deficiencies by enlargement of the thyroid is evident from the investigations of Welch in Montana. On this subject he[2] says:

> Goiter in poultry is very common. Flocks with a very high percentage of it have been noted in goiterous areas. The enlarged thyroid, frequently as large as the thumb, is entirely concealed by the feathers, and so far we have not observed any bad effect on the health of these fowls. The owners of goitered flocks have always reported that egg production and general health were normal. We have attempted to determine whether congenital goiter existed among newly hatched chicks but have been unable to get the material to study. No losses have been reported, at any rate, from this cause.

A detailed report of a case of simple colloid goiter in poultry has been reported by Kernkamp.[3] In Minnesota, goiter in poultry, according to this report, is extremely uncommon.

In the case of obvious goiter, the medicinal use of iodine is justified and may be expected to give relief on the basis of experimental investigations on other animals. But in those localities of the country, including Illinois, in which goiter in farm animals is not endemic and is in fact of rare occurrence, the necessity or the wisdom of supplementing farm rations with some form of iodine is not self-evident and can be determined only by controlled experimental investigations. At present there is a lack of experimental evidence on the growth-promoting value of an iodine supplement to the rations of poultry. This situation prompted the experiment reported in this paper.

The experiment represents an attempt to extend the paired-feeding method to groups of three, the food intakes of each triplet being kept approximately the same. The result was not entirely successful, but nevertheless the information obtained seems to warrant publication.

## EXPERIMENTAL PROCEDURE

Eighteen White Leghorn chicks, about 6½ weeks of age, were divided into six groups of three birds each in such a way that the birds in each triplet were of the same sex and of about the same weight. Five of the triplets were made up of pullets and the sixth of cockerels. At the beginning of the experiment the birds ranged in weight from 230 to 320 gm., averaging 270 gm.

All birds were fed the same basal ration[4] of ground yellow corn 65 parts, wheat bran 10 parts, middlings 10 parts, tankage 10 parts, steamed bone meal 3 parts, sodium chloride 1 part, and charcoal 1 part.

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[1] Received for publication Feb. 1, 1930; issued July, 1930.

[2] WELCH, H. GOITER IN FARM ANIMALS. Mont. Agr. Expt. Sta. Bul. 214, 26 p., illus. 1928.

[3] KERNKAMP, H. C. H. GOITER IN POULTRY. Jour. Amer. Vet. Med. Assoc. 67: 223–228, illus. 1925.

[4] A composite sample of this ration, covering the entire period of the experiment, was analyzed with the following results: Dry substance, 92.43 per cent; crude protein, 16.31 per cent; nitrogen-free extract, 54.59 per cent; crude fiber, 3.44 per cent; ether extract, 10.99 per cent; and ash, 7.10 per cent.

TABLE 1.—*Body weights, weekly gains or losses, and food consumption of growing chicks as affected by feeding potassium iodide at two levels[a] as a mineral supplement*

| Item | Group 1 | | | Group 2 | | | Group 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Check bird | Bird fed 0.5 mgm. KI | Bird fed 1 mgm. KI | Check bird | Bird fed 0.5 mgm. KI | Bird fed 1 mgm. KI | Check bird | Bird fed 0.5 mgm. KI | Bird fed 1 mgm. KI |
| Final weight...gm.. | 1,110 | 1,120 | 560 | 510 | 1,020 | 1,070 | 1,060 | 1,120 | 1,100 |
| Initial weight..do.... | 320 | 310 | 310 | 290 | 280 | 290 | 270 | 270 | 270 |
| Total gain.....do.... | 790 | 810 | 250 | 220 | 740 | 780 | 790 | 850 | 830 |
| Test period...days.. | 98 | 98 | 21 | 21 | 98 | 98 | 98 | 98 | 98 |
| Average daily gain....gm.. | 8.06 | 8.26 | (11.90) | (10.48) | 7.55 | [b]7.96 | 8.06 | 8.67 | 8.47 |
| **WEEKLY GAINS** | | | | | | | | | |
| Week: | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 110 | 95 | 110 | 70 | 70 | 70 | 100 | 80 | 100 |
| 2 | −20 | 65 | 60 | 70 | 60 | 80 | 45 | 40 | 80 |
| 3 | 170 | 60 | 80 | 80 | 70 | 80 | 75 | 60 | 60 |
| 4 | 50 | 100 | ..... | ..... | 50 | 70 | 50 | 30 | 20 |
| 5 | −40 | −50 | ..... | ..... | −20 | −30 | −60 | −40 | −40 |
| 6 | 130 | 160 | ..... | ..... | 130 | 160 | 210 | 230 | 190 |
| 7 | 50 | 50 | ..... | ..... | 40 | 40 | −60 | −60 | −80 |
| 8 | 50 | 50 | ..... | ..... | −10 | 60 | 60 | 70 | 140 |
| 9 | 50 | 60 | ..... | ..... | 150 | 120 | 150 | 150 | 100 |
| 10 | 80 | 90 | ..... | ..... | 50 | 60 | 100 | 60 | 110 |
| 11 | 50 | 40 | ..... | ..... | −30 | 40 | 0 | 60 | 0 |
| 12 | 50 | 30 | ..... | ..... | 80 | 120 | 80 | 70 | 80 |
| 13 | 30 | 40 | ..... | ..... | 70 | −200 | 30 | 80 | 60 |
| 14 | 30 | 20 | ..... | ..... | 30 | 110 | 10 | 20 | 10 |
| Total feed eaten | 5,335 | 5,335 | 950 | 1,050 | 6,195 | 6,195 | 6,290 | 6,300 | 6,040 |
| Average daily ration | 54.4 | 54.4 | 45.2 | 50 | 63.2 | 63.2 | 64.2 | 64.3 | 61.6 |
| Feed per gram gain | 6.75 | 6.58 | (3.80) | (4.77) | 8.37 | 7.94 | 7.96 | 7.41 | 7.27 |

| Item | Group 4 | | | Group 5 | | | Group 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Check bird | Bird fed 0.5 mgm. KI | Bird fed 1 mgm. KI | Check bird | Bird fed 0.5 mgm. KI | Bird fed 1 mgm. KI | Check bird | Bird fed 0.5 mgm. KI | Bird fed 1 mgm. KI |
| Final weight...gm.. | 920 | 950 | 1,010 | 930 | 1,020 | 1,040 | 1,240 | 1,120 | 1,140 |
| Initial weight..do.... | 260 | 260 | 260 | 240 | 230 | 260 | 260 | 250 | 230 |
| Total gain.....do.... | 660 | 690 | 750 | 690 | 790 | 780 | 980 | 870 | 910 |
| Test period...days.. | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 | 98 |
| Average daily gain....gm.. | 6.73 | 7.04 | 7.65 | 7.04 | 8.06 | 7.96 | 10.00 | 8.88 | 9.28 |
| **WEEKLY GAINS** | | | | | | | | | |
| Week: | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* | *Gm.* |
| 1 | 70 | 85 | 65 | 70 | 60 | 120 | 140 | 35 | 70 |
| 2 | 50 | 55 | 70 | 60 | 70 | −20 | 90 | 75 | 140 |
| 3 | 90 | 50 | 35 | 50 | 40 | 50 | 75 | 110 | 50 |
| 4 | 50 | 80 | 65 | 80 | 60 | 10 | 115 | 90 | 90 |
| 5 | −30 | −50 | −5 | −10 | 10 | −10 | −90 | −10 | 0 |
| 6 | 180 | 140 | 110 | 120 | 140 | 160 | 195 | 130 | 100 |
| 7 | 25 | 40 | 40 | 70 | 60 | 70 | 75 | 20 | 70 |
| 8 | −25 | −10 | 40 | 20 | 40 | −20 | 10 | 30 | 100 |
| 9 | 130 | 110 | 50 | 100 | 60 | 150 | 120 | 130 | 50 |
| 10 | 60 | 60 | 50 | 60 | 80 | 90 | 70 | 120 | 90 |
| 11 | −10 | 0 | 40 | −10 | 30 | 30 | 60 | 30 | 0 |
| 12 | 10 | −40 | 60 | 50 | 40 | 90 | 30 | 120 | 100 |
| 13 | 10 | 20 | 40 | 40 | 50 | −20 | 60 | −40 | 40 |
| 14 | 50 | 150 | 90 | −10 | 50 | 80 | 30 | 30 | 10 |
| Total feed eaten | 5,475 | 5,475 | 5,475 | 6,485 | 6,485 | 6,565 | 6,350 | 6,270 | 6,320 |
| Average daily ration | 55.9 | 55.9 | 55.9 | 66.2 | 66.2 | 67.0 | 64.8 | 64.0 | 64.5 |
| Feed per gram gain | 8.39 | 7.93 | 7.30 | 9.40 | 8.21 | 8.42 | 6.48 | 7.21 | 6.95 |

[a] The feeding was in each case at the rate of 0.5 or 1 mgm. potassium iodide daily per 100 gm. body weight.
[b] This bird laid 5 eggs during the last 2 weeks of the experiment. The total weight of the eggs was 185 gm.

The birds were fed individually in cages containing approximately 4 square feet of floor space. The food intake of each of the three birds in each triplet was kept the same, or very nearly so, the amount offered being determined by the bird that ate the least. Grit and distilled water were available to all birds at all times. All birds were irradiated daily for 10 to 15 minutes with ultra-violet light from a mercury vapor quartz lamp.

In each triplet, one of the three birds received only the basal ration, a second was given, in addition to the basal ration, 0.5 mgm. of potassium iodide daily per 100 gm. of body weight, while the ration of the third bird was supplemented daily with 1 mgm. of potassium iodide per 100 gm. of body weight. The potassium iodide, in the form of an aqueous solution containing 1 mgm. of the salt per cubic centimeter, was introduced directly into the crop from a burette fitted with a rubber tube of small bore.

The birds were weighed each week, and the experiment was continued for 14 weeks. Two birds, one the control bird in Group 2, and the other the bird receiving the larger dosage of iodide in Group 1, died after three weeks from unknown causes.

## EXPERIMENTAL DATA

The results of the experiment have been summarized in Table 1. Of the 5 possible comparisons of total gains between birds receiving the smaller dosage of iodide and their controls, 4 favored the iodide bird and 1 favored the control. Of the 4 possible comparisons between birds receiving the higher dosage of iodide and their controls, 3 favored the iodide bird and 1 the control. Of the 5 possible comparisons between birds receiving the two dosages of iodide, 3 favored the bird receiving the larger dosage.

With only four or five comparisons possible, any division of the results, such as was thus obtained in all cases, is sufficient to prevent the formulation of a positive conclusion as to the effect of potassium iodide on growth, since such an outcome may reasonably have occurred from chance only.

The essential negativity of the results is further revealed by making comparisons between the weekly gains of comparable birds. There are 73 possible comparisons between birds getting the lower dosage of iodide and their controls, 37.5 of which favored the iodide bird; of 62 comparisons between birds receiving the higher dosage of iodide and their controls, 33 favored the iodide birds; while of 73 comparisons between the two birds in each triplet receiving iodide, 39.5 favored the bird getting the higher dose. These results are so near the ideal outcome if chance alone operated, i. e., 36.5, 31, and 36.5, respectively, that the operation of the deliberately imposed experimental condition need not be assumed.

## SUMMARY

In a feeding experiment involving 18 White Leghorn chicks, individually fed so that birds whose gains were to be compared were of approximately the same initial weight and received approximately the same amount of food, no evidence was obtained that a supplement of potassium iodide at the rate of 0.5 mgm. or of 1.0 mgm. daily per 100 gms. of body weight in any way influenced the rate of growth.

# A COMPARATIVE STUDY OF THE PROTECTIVE VALUE OF CERTAIN FABRICS IN STILL AND MOVING AIR[1]

By KATHERINE HESS, *Assistant Professor, Department of Clothing and Textiles;* E. V. FLOYD, *Professor, Department of Physics;* and LILIAN BAKER, *Professor and Head, Department of Clothing and Textiles, Kansas Agricultural Experiment Station*

## INTRODUCTION

Clothing made from wool fabrics is generally regarded as affording the body more protection than that made from cotton. This indicates that wool is a poorer conductor of heat than cotton. There are available data to prove that the wool and the cotton fibers differ greatly in their capacity to conduct heat. (See footnote 10.) Therefore, if wool fabrics are warmer than cotton there must be other influencing factors than the heat conductivity of the fibers themselves.

The heat conductivity of a fabric may differ greatly from that of the fiber from which it is made. This may be due to a number of factors, such, for example, as the construction of the fabric, the finish, and in the case of a rough surface, the position of the nap or pile in relation to the body.

The purpose of this study was to compare the relative protective ratios of various fabrics as found in still air and in wind of different velocity.

## REVIEW OF LITERATURE

Many investigators have studied the protection afforded the body through clothing. Count Rumford performed experimental work of this nature in the latter part of the eighteenth century. In this work, as reported by Williams,[2] Rumford compared the protective value of different fibers when loosely and when tightly packed around a body. His method was to inclose the bulb of a thermometer in a known weight of fibers, raise the mass to 190° F., and note the time required for the mercury to drop a given number of degrees. This work proved that small quantities of fibers holding air in their meshes offered a greater protection from heat loss than a larger quantity of fibers packed into the same space.

LeFerve [3] made a study of the protective value of clothing fabrics in currents of air at different temperatures. In this work a measured quantity of air at a known speed and temperature was driven through a calorimeter past a clothed or unclothed human body. The temperature of the air was taken before it reached the body and as it left it. LeFerve concluded that the unclothed body lost more heat in a given time than the clothed body, and that the lower the environmental temperature and the greater the speed of the wind, the greater was the loss of heat.

Bergonie and Coulier [4] determined the time required for a vessel of warm water covered and uncovered to cool 10° C. From their

[1] Received for publication Feb. 3, 1930; issued July, 1930. Contribution No. 9 from the department of clothing and textiles, Kansas Agricultural Experiment Station.

[2] WILLIAMS, M. PHILOSOPHY OF CLOTHING. Edinburgh and Glasgow. 1890.

[3] BURNS D. AN INTRODUCTION TO BIOPHYSICS. (With a foreword by D. Noël Paton). 435 p., illus. New York. 1921.

[4] BURNS, D. Op. cit.

work they concluded that the amount of air held in a fabric determines to a great extent its protective value. Krieger, according to Pettenkofer,[5] reported experiments conducted with single and double layers of fabrics. His method was to cover a metal vessel of hot water with a fabric and to determine the time required for the water to cool through a given number of degrees. He concluded that it was not the kind of fiber or its weight but the texture and the volume which are the principal causes of differences in heat retention by fabrics.

Rubner[6] contributed valuable data on the heat conductivity of clothing fabrics. His work included the influence of the moisture in a fabric on its protective value. He found that moisture lowered the protective value of clothing.

Caton[7] conducted experiments to determine the effect of weave, color, and fiber on the heat-retaining properties of fabrics. Bottles

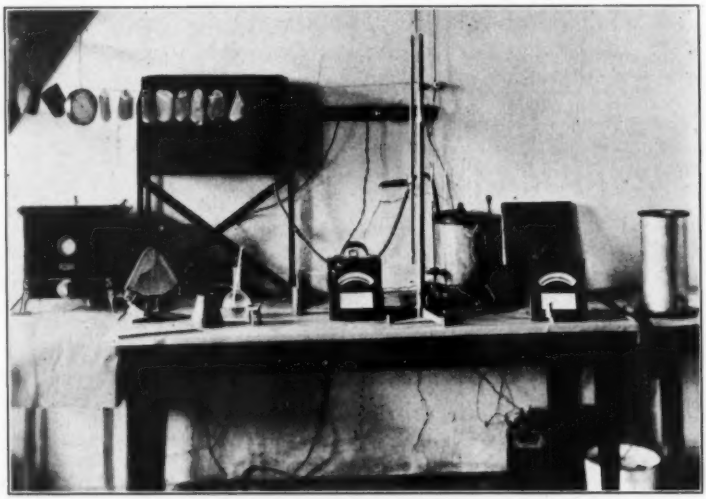

FIGURE 1.—Special apparatus used in the study of the protective value of fabrics

of warm water were covered with the fabrics under study and the time required for the bottles to cool was compared. She concluded that moisture in a fabric increased its ability to prevent loss of heat from a body.

Clark[8] measured the protective value of certain clothing fabrics. The study included analyses of the fabrics used and determination of the electrical energy that would be necessary to keep a fabric-covered body at a temperature of 36° C. under rigidly controlled conditions of relative humidity and temperature. Thirteen fabrics were employed in this work. The conclusions drawn were that the protective ratio of a clothing fabric can be determined in terms of electrical

[5] PETTENKOFER, MAX VON. THE RELATION OF THE AIR TO THE CLOTHES WE WEAR, THE HOUSE WE LIVE IN AND THE SOIL WE DWELL ON. [Typewritten copy on file, Kansas State Agricultural College, Manhattan.]

[6] RUBNER, M., GRUBER M. VON, FICKER, M., editors. HANDBUCH DER HYGIENE, UNTER MITWIRKUNG VON R. ABEL, J. BOETHKE [u. a.]. v. illus. Leipzig.

[7] CATON, F. THE HYGIENE OF WOMEN'S UNDERWEAR. (Thesis. Univ. of Missouri). 1920.

[8] CLARK, F. R. THE PROTECTIVE VALUE OF CERTAIN CLOTHING FABRICS. [Masters thesis. Copy on file Kansas State Agricultural College, Manhattan.] 1925.

energy; that the greater the percentage of moisture in a fabric the lower is the protective value; and that there was in the fabric tested no consistent correlation between the protective ratio and the mass per unit area of the fabric.

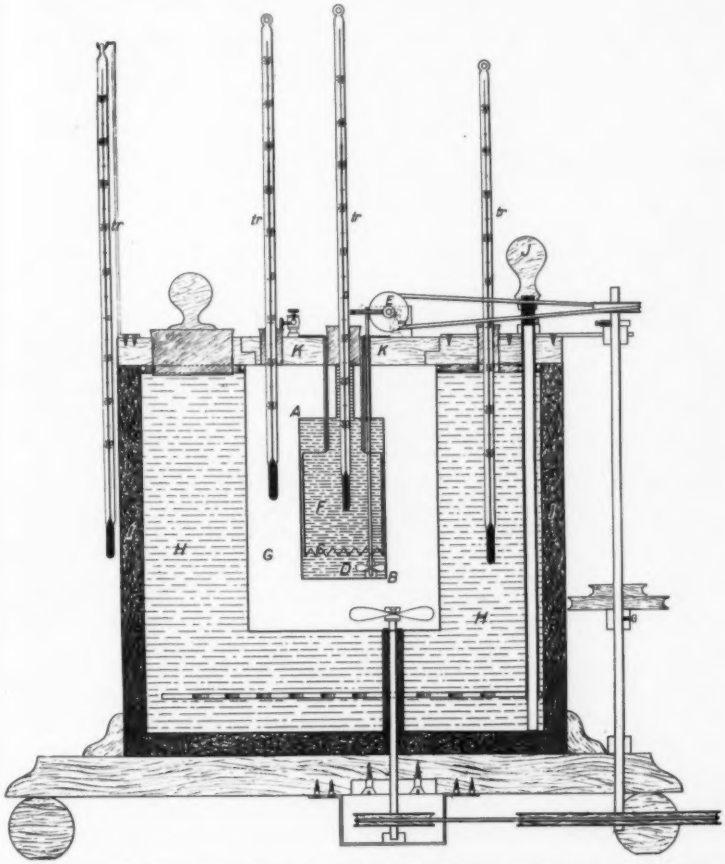


FIGURE 2.—Detailed drawing of calorimeter: A–B, Oil-filled copper cylinder 2 inches in diameter and 4 inches long; C, heating coil terminating in binding posts on calorimeter top; D, a 4-blade stirrer operated by worm gear E and the system of pulleys, shafts, and belts shown in the diagram; F, transformer oil filling the copper cylinder; G, air maintained in slow motion by the small electric fan as shown; H, water jacket surrounding the air calorimeter; J, stirrer for water in jacket; K, wooden top containing rubber gaskets which seal the air calorimeter; *tr*, thermometers for reading temperatures of room, water, air in the calorimeter, and oil

## EXPERIMENTAL METHODS

The apparatus for the present study was constructed from the same design and the method of procedure was identical with that used by Clark.[9] The fabric analyses as reported by Clark were accepted in this work. An air-tight box in which the relative humidity

[9] CLARK, F. R. See footnote 8.

and temperature were easily controlled aided greatly in conditioning the fabrics.

The method used was that of compensating electrically the heat loss from an oil-filled copper cylinder placed in a well-insulated calorimeter. The special apparatus is shown as a whole in Figure 1 and the calorimeter in detail in Figure 2.

The experiments were performed in a room equipped in such a manner that the temperature and relative humidity of the air could be measured and regulated. A relative humidity of $40 \pm 1$ per cent and a temperature of $25° \pm 1°$ C. were used for this work. The fabrics were kept in the constant-humidity box under these conditions, and air of this relative humidity and temperature was blown into the air chamber of the calorimeter immediately before each experiment. The chamber was practically air-tight, and the temperature was held constant by means of the water jacket. The oil in the cylinder and the air surrounding it were stirred just enough to keep the temperature even throughout. The temperatures were read from thermometers accurate to 0.1° C.

A 6 to 9 volt storage battery was wired through an adjustable resistance to the heating element. By means of the adjustable resistance, an ammeter, and voltmeter the amount of electrical energy necessary to compensate for the heat losses from the oil-filled cylinder to its environment could be determined. It was found possible by this means to so accurately compensate for the heat loss that no perceptible change in temperature could be detected with the thermometer used. The electrical energy required to maintain the bare cylinder at 36° C. was recorded. The copper cylinder was then covered with the fabric under study and replaced in the calorimeter. The compensating energy was again determined for the fabric-covered cylinder.

The ratio of the compensating energy of the bare cylinder to that of the clothed cylinder was taken as the ratio of protection for the fabric.

The following fabrics commonly used in clothing were studied: Canton flannel, nap in and nap out; knit cotton underwear, knit infant's vest, wool and cotton, navy-blue flannel, and gray astrakhan, pile in and pile out.

The analyses of these fabrics included the kind of fiber, method of construction, weight per square yard, picks and ends, breaking strength, twist per inch of yarn, and the mass per unit area. These were determined for the dry fabrics in grams per square meter and in ounces per square yard.

The percentage of enmeshed air in the fabrics was determined by means of the thickness, the total volume, and the volume of the air.[10] The thickness of the fabric containing the enmeshed air was found in the following manner: By means of a micrometer microscope the thickness of the fabric was measured, and the following weights were then applied over a space 1 inch square: 2.12, 4.12, 10.12, 20.12, 40.12, and 160.12 gm. A curve was plotted from these weights and measurements. This curve was extended by

[10] MALONEY, M. A. THE VOLUME OF ENMESHED AIR AS A FACTOR IN THE PROTECTIVE VALUE OF CLOTHING. [Unpublished manuscript. Clothing and Textiles Dept., Kansas State Agricultural College, Manhattan.]

extrapolation to where it intercepted the zero line. The values thus determined were taken as the thickness of the fabric containing the volume of enmeshed air with no weight applied.

The amount of air in 100 sq. cm. of fabric was determined by measuring the volume of water replaced by the fabric after all the air that could be pressed out had been removed. The total volume, or volume of the fabric and air, minus the volume of the fabric, gave the volume of enmeshed air. The percentage of enmeshed air was found by dividing the volume of enmeshed air by the total volume.

TABLE 1.—*Quantity of enmeshed air, thickness of fabric, mass in ounces per square yard, protective ratio, and protective values in per cent of various fabrics when tested in still air and in winds of differing velocities*

| Fabric | Thickness | Mass | Enmeshed air | Speed of wind | Protective ratio | Protection |
|---|---|---|---|---|---|---|
| | *Mm.* | *Oz. per sq. yd.* | *Per cent* | *Miles per hour* | | *Per cent* |
| Canton flannel (nap side in) | 2.22 | 10.00 | 87.9 | 0 | 1.15 | 13.74 |
| White knit goods (wool and cotton) | 2.1 | 11.31 | 83.77 | | 1.18 | 15.47 |
| White knit underwear (all cotton) | 1.92 | 11.14 | 81.96 | | 1.18 | 15.64 |
| Blue flannel (wool) | 1.03 | 4.71 | 89.93 | | 1.20 | 17.06 |
| Canton flannel (nap side out) | 2.22 | 10.00 | 87.9 | | 1.24 | 19.84 |
| Gray astrakhan (pile side in) | 4.15 | 15.70 | 89.25 | | 1.33 | 24.86 |
| Gray astrakhan (pile side out) | 4.15 | 15.70 | 89.25 | | 1.39 | 28.40 |
| Canton flannel (nap side in) | | | | 2.2 | 1.16 | 14.52 |
| White knit goods (wool and cotton) | | | | | 1.210 | 17.30 |
| White knit underwear (all cotton) | | | | | 1.213 | 17.53 |
| Blue flannel (wool) | | | | | 1.23 | 18.88 |
| Canton flannel (nap side out) | | | | | 1.303 | 23.28 |
| Gray astrakhan (pile side in) | | | | | 1.402 | 28.68 |
| Gray astrakhan (pile side out) | | | | | 1.43 | 30.11 |
| Canton flannel (nap side in) | | | | 3 | 1.18 | 15.47 |
| White knit goods (wool and cotton) | | | | | 1.28 | 21.80 |
| White knit underwear (all cotton) | | | | | 1.29 | 22.78 |
| Blue flannel (wool) | | | | | 1.31 | 23.07 |
| Canton flannel (nap side out) | | | | | 1.45 | 30.92 |
| Gray astrakhan (pile side in) | | | | | 1.49 | 33.04 |
| Gray astrakhan (pile side out) | | | | | 1.59 | 37.04 |
| Canton flannel (nap side in) | | | | 4.5 | 1.25 | 20.49 |
| White knit goods (wool and cotton) | | | | | 1.4 | 28.51 |
| White knit underwear (all cotton) | | | | | 1.41 | 28.93 |
| Blue flannel (wool) | | | | | 1.42 | 30.12 |
| Canton flannel (nap side out) | | | | | 1.52 | 38.56 |
| Gray astrakhan (pile side in) | | | | | 1.56 | 36.04 |
| Gray astrakhan (pile side out) | | | | | 1.72 | 42.03 |
| Canton flannel (nap side in) | | | | 8.5 | 1.26 | 21.29 |
| White knit goods (wool and cotton) | | | | | 1.42 | 29.81 |
| White knit underwear (all cotton) | | | | | 1.50 | 33.33 |
| Blue flannel (wool) | | | | | 1.49 | 35.45 |
| Canton flannel (nap side out) | | | | | 1.63 | 38.71 |
| Gray astrakhan (pile side in) | | | | | 1.81 | 44.69 |
| Gray astrakhan (pile side out) | | | | | 1.82 | 45.12 |

## EXPERIMENTAL DATA

The work in still air indicates that the percentage of enmeshed air in a fabric determines to a great extent the protective ratio of that fabric, or the protection that a fabric will afford in preventing loss of heat from a body.

If the protective ratio of the fabric in still air is a function of the enmeshed air, it is important to know the effect of air in motion on this ratio. To determine this point an investigation was made to test certain of these same fabrics in air currents of different velocities. The oil cylinder, fabric capsules, and thermometers used in the first part of the work were employed in this study.

In order to provide air currents a wind tunnel was constructed which contained a motor-driven fan, the oil-filled copper cylinder, and the necessary thermometers, hygrometer, humidifying mechanism, and anemometer. A longitudinal section of this apparatus is shown in Figure 3.

The tunnel (fig. 3) was constructed of six joints and four elbows of 7-inch stovepipe. These were joined in such a manner as to provide an air-tight continuous path for the air current. One of the sections of pipe (A) was insulated and fitted to receive thermometers, the hygrometer, and the oil-filled copper cylinder. Another section (B) was jacketed in such a manner as to permit a circulation of water around the pipe so that the temperature of the air in the tunnel could be controlled. Air currents in the tunnel were created by means of a 4-blade fan (C) placed near one elbow and driven by an electric motor (D) located on the outside. The velocity of the moving air was deter-

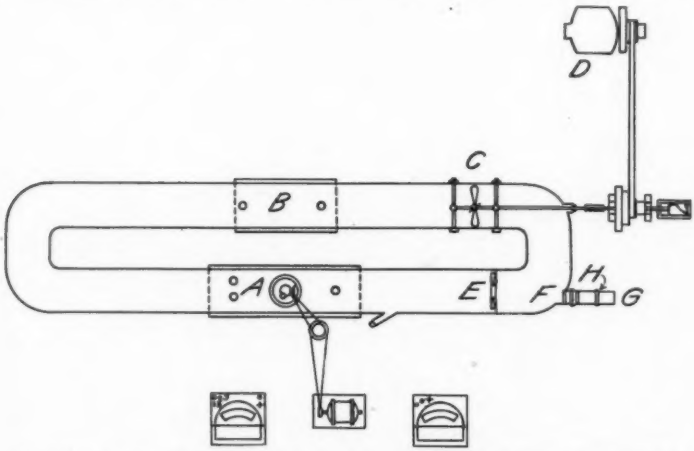


FIGURE 3.—Longitudinal section of wind tunnel: A, Insulated section containing thermometers, hygrometer, and oil-filled copper cylinder; B, section of tunnel surrounded with water jacket; C, 4-blade fan; D, electric motor to drive fan; E, anemometer; F, glass window; G, flash light; H, magnifying glass; I, wet-bulb thermometer; J, oil-filled copper cylinder. Scale, three-fourths inch to 1 foot

mined by means of a 6-inch Keuffel and Esser anemometer (E) placed in the tunnel in such a manner that its readings could be taken through a glass window (F) in the wall of the tunnel. This was done with the aid of a flash light (G) and a magnifying glass (H). Figure 4 shows the apparatus as first set up. The small motor was later changed for a quarter-horse power direct-current motor. The different speeds were obtained by means of a combination of pulleys. It was possible to obtain seven different wind speeds varying from 2 to 18 miles per hour. Only four of these were used.

Experiments showed that with a given combination of pulleys the wind current varied not more than 0.6 mile per hour. The relative humidity of the air in the tunnel could be easily controlled. The temperature of the tunnel was best controlled by regulating that of the room. If the temperature of the room could not be controlled, that of the air in the tunnel was regulated with difficulty by circulating water through the jacket covering one section of the pipe.

The air in the tunnel was set in motion and regulated to $40 \pm 1$ per cent relative humidity at 25° C. The procedure for testing the fabrics was the same as that followed in the first part of the work.

Table 1 shows the percentage of enmeshed air, the thickness of the fabric, mass in ounces per yard, and the protective ratio and protective value in terms of percentage for the fabrics in still air and in air moving at the rate of 2.2, 3, 4.5, and 8.5 miles per hour. The variation in velocity of the wind due to the variation in electrical current made it somewhat difficult to secure closely agreeing checks and resulted in slight irregularities in the data. The fabrics are listed in the order of their protective ratio. It will be noticed that this order is the same both in still air and in air moving at the speeds tested. This does not mean that the electrical energy required to compensate for heat loss decreases as the wind speed increases, but that the electrical energy required to compensate for the heat loss of the unclothed body

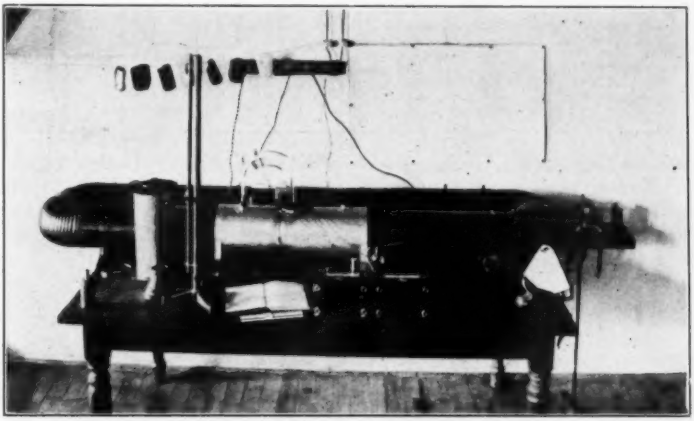

FIGURE 4.—Wind tunnel and auxiliary apparatus

increases more rapidly as the wind speed increases than does that for the clothed body, resulting in a greater difference and a higher protective ratio.

In every case the Canton flannel (all cotton) and gray astrakhan (knit cotton back and wool pile) increased in protective ratio when the nap or pile was exposed to the wind or still air and the plain surface was next to the body.

Placing the pile or nap on the inside lowered the insulation possibly by (1) exposing the larger mass of the fiber of the fabric to the lower temperature, and (2) crushing the pile or nap by contact with the body and thus reducing the amount of enmeshed air in the fabric.

Table 2 shows the rating of the fabrics on the basis of their protective ratio, percentage of enmeshed air, thickness, and mass. It will be noticed that wool flannel ranks lowest in mass and thickness, fifth in percentage of enmeshed air, and fourth in protective ratio. Canton flannel is second in mass, fourth in thickness, and third in percentage of enmeshed air. This fabric ranks lowest in protective ratio when the nap is next to the body and fifth when the nap is out.

It would be necessary to handle more fabrics and to compare certain wool fabrics with other wool fabrics and certain cotton fabrics with other cotton fabrics in order to determine the relationship of the percentage of enmeshed air to the protective ratio for a group.

TABLE 2.—*Numerical ratings of protective value, percentage of enmeshed air, thickness, and mass of various fabrics listed in order of protective value, beginning with the least protective*

| Fabric | Protective ratio | | Enmeshed air | Thickness | Mass |
|---|---|---|---|---|---|
| | Still air | Moving air | | | |
| | | | *Per cent* | *Mm.* | *Oz. per sq. yd.* |
| Canton flannel (all cotton; nap turned in) | 1 | 1 | 3 | 4 | 2 |
| White knit goods (wool and cotton) | 2 | 2 | 2 | 3 | 3 |
| White knit (all cotton) | 3 | 3 | 1 | 2 | 4 |
| Wool flannel (all wool) | 4 | 4 | 5 | 1 | 1 |
| Canton flannel (nap turned out) | 5 | 5 | 3 | 4 | 2 |
| Gray astrakhan (knit cotton back, wool pile; pile turned in) | 6 | 6 | 4 | 5 | 5 |
| Gray astrakhan (pile turned out) | 7 | 7 | 4 | 5 | 5 |

Interesting problems suggested by the study are: The determination of the heat conductivity of certain types of clothing fabrics; the effect of fitting, the effect of layers, and the effect of relative humidity and temperature on the protective value of clothing fabrics.

## CONCLUSIONS

The following conclusions are drawn from the data collected under the conditions of this study:

The rating of the fabrics on the basis of increasing protective ratio was found to be the same in still air and in air moving at 2.2, 3, 4.5, and 8.5 miles an hour.

The protective ratio of a fabric increases as the speed of the wind increases. An increase from 0 to 8.5 miles an hour corresponds to an average increase in protection of 5.67 per cent.

When made into closely fitting covers, fabrics having a pile or nap afforded greater protection when the smooth surface was next to the body. This was found to be true in determinations made in both still and moving air.

# THE LIMITS OF ERROR OF THE BABCOCK TEST FOR CREAM [1]

By W. H. MARTIN, *Professor of Dairy Husbandry*, A. C. FAY, *Associate Professor of Bacteriology*, and K. M. RENNER, *Instructor of Dairy Husbandry, Kansas Agricultural Experiment Station*

## INTRODUCTION

Since its invention in 1890 the Babcock test for milk and cream has superseded all other fat tests in the United States and Canada. In this country it is now recognized by the Association of Agricultural Chemists as a standard method for butterfat determination. In Australia, New Zealand, South Africa, and the Argentine the Babcock test is used almost exclusively.

Recognizing the necessity of protecting the financial interests of both sellers and buyers of butterfat, most States have passed laws which provide for the examination of cream testers to determine their proficiency. Most of these laws also stipulate the use of accurate glassware, weights, and other equipment, which must be approved. A system of periodic inspection by State authorities aims to insure efficient and honest testing.

As used to-day, the Babcock test is essentially the same as it was when introduced. The accuracy of the test has been checked from time to time by various investigators. Webster (*12, p. 17*)[2] was one of the first to determine the value of the meniscus. He concluded that readings taken at the top, middle, or bottom of the meniscus did not give true percentages of butterfat as determined by the gravimetric extraction method. It remained for Hunziker and his associates (*6*) in 1910 to suggest glymol as a means of eliminating the meniscus in reading cream tests. A few years later Spitzer and Epple (*11*), and Doan and his coworkers (*2*), in comparing the cream tests made by the Babcock and the fat-extraction methods, reported that readings with glymol approached closely the chemical analysis.

Another phase of cream testing which has received some attention is the effect of souring on the test of cream. Farrington (*3, p. 5*), in studying this problem, reported no difference in the tests from a can of cream before and after souring. Hunziker and his associates (*5*) also demonstrated conclusively that there was no increase in the test of cream after souring. Their work showed, however, that in some instances where cans of cream were allowed to stand uncovered for a considerable time in a warm place there was a slight increase in the cream test due to evaporation.

Comparisons have been made by Dahlberg and his coworkers (*1, p. 29*), in which the Babcock test was checked against the Roese-Gottlieb and the Gerber methods. This work showed that duplicate Roese-Gottlieb tests made in the same laboratory generally agreed

[1] Received for publication Mar. 5, 1930; issued July, 1930. Contribution No. 68 from the department of dairy husbandry and No. 119 from the department of bacteriology, Kansas Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 159.

within 0.16 per cent and tests made in different laboratories did not vary more than 0.5 per cent. The average of all the tests made by the Babcock and the Gerber methods was accurate to within 0.4 per cent or less. Ross and McInerney (*9*) found in testing 64 samples of cream by the Babcock and ether-extraction methods that 35 tests checked within 0.29 and only 8 varied more than 0.5 per cent. A conclusion was reached by Siegmund and Craig (*10*) that Babcock tests for cream gave readings slightly higher than the ether-extraction method due to the inclusion of some water and acid in the fat column. Nelson (*8*), in comparing 2,000 Babcock tests on market milk, found that the probable error of $\pm 0.02$ per cent was due mainly to the method of reading.

## PURPOSE OF THIS INVESTIGATION

Considering the stipulations of the various State laws relative to the limits of error of fat tests, it seemed advisable to ascertain the normal fluctuation to be expected in applying the Babcock method. Obviously, it would be unfair to require an apprentice tester to attain a degree of accuracy that can not be attained by an experienced technician. On the other hand, if the limits of error permitted by any State law are too great, the intent of the law is defeated by allowing careless and incompetent testers in the field.

The purpose of this investigation, then, is to measure the degree of normal fluctuation that may be expected in fat testing, with the ultimate aim of creating a better basis of judging the permissible limits of error.

In this discussion the term "error" means any deviation from the true fat content as far as it is obtainable by the Babcock method. For a given can of cream there is only one true value for the fat content, and any other value is in error whether the deviation be due to careless technic or to factors beyond the control of the operator. It is reasonable to expect that several samples taken from the same can of cream may contain slightly different percentages of fat. The degree of this error, of course, will be largely dependent on the thoroughness with which the cream has been agitated, but in any case some variation may be expected. These variations may result from the cream being too sour, thus rendering it difficult to procure a fair sample. Other errors undoubtedly result from imperfect weighing of the 9 gm. sample and from reading imperfect tests in which the fat column has been too badly charred by the acid. Individuals may read the same test differently. The extent of this divergence depends somewhat on the degree of experience of those reading the tests, but varies also among those who have had extensive experience.

This experiment has been organized so as to segregate some of these sources of error in order to measure their relative magnitude.

## PLAN OF THE EXPERIMENT

The experiments reported in this paper have been so arranged as to measure the expected limits of error of the fat tests, the variation in readings by several persons, the error which results from careless or hasty reading, and the effect of souring on the test.

A large number of Babcock determinations were made by an experienced tester on each of two 10-gallon cans of cream, and the tests were read by five persons. From the data collected it was possible to determine the extent and degree of variability of this test in the hands of a skilled operator. Mojonnier (*7, p. 11*) tests on these samples of cream also permitted a comparison of the Babcock and ether-extraction methods. Two other groups of identical samples were submitted to three laboratories in such a way as to eliminate the psychological factor of knowing the identity of the samples. Statistical analysis of the data not only reveals the extent and degree of variation as affected by the various segregated factors, but gives some basis of judging the limits within which normal variation may be expected.

In taking all the samples for the experiments reported in this paper, unusual precautions were used in order to render the replicate samples as nearly identical as possible. The cream to be sampled was first poured back and forth from one can to another 10 times, and kept constantly in motion with a stirring rod while 8 to 10 pint samples were removed with a dipper. The cream was again poured back and forth 10 times before another group of 8 to 10 samples was removed. This process was repeated until the required number of samples was obtained.

The Babcock test was made according to the method of the American Dairy Science Association (*4*). All test bottles used were calibrated. The Mojonnier (*7*) tests were run according to the directions which are supplied with the machine.

## RESULTS

### VARIATION IN THE READINGS OF DIFFERENT PERSONS

#### Sweet Cream

Eight 1-pint samples of sweet cream were taken according to the method previously described, and 12 replicate Babcock tests were made on each sample. Each test bottle was passed down a line of five readers, each of whom read and recorded his test privately without knowledge of the value given by other readers. The readings were made in such a manner that not more than one minute elapsed between the first and last readings on any test bottle. There were 96 tests and 456 readings made on this can of sweet cream. (One reader failed to read the tests on two of the samples.)

Some variation may be expected in the results of this method even when in the hands of a skilled operator or an experienced reader. The values established by the mean test plus or minus 3.2 times the probable error were arbitrarily accepted as marking the upper and lower limits between which a tester or reader is practically certain (30 to 1 chance) that any average of duplicate determinations will fall. That is to say, the operator can be reasonably sure that in any reading outside of these limits the deviation is due to some factor other than chance variation. In the subsequent discussion of the data the limits established by a 30 to 1 chance will be regarded as the limits of practical certainty.

Table 1 shows the minimum, maximum, and mean readings reported on each of eight samples as read by five readers. For example, of

the 12 tests on sample 1 read by five people, the lowest test reported by anyone was 41.50, the highest 42.50, and the average of the 60 readings 41.97 per cent. Similarly, Table 1 shows the minimum, maximum, and mean of the 456 readings on all samples to be 41, 42.50, and 41.76 per cent respectively.

It must be borne in mind that the extreme readings are based on single tests and are not the averages of duplicate determinations. Since the average of the 456 readings of 96 tests, 41.76 per cent, is as near to the true test on this can of cream as these data will afford, it may be seen that the normal variation of single tests may account for readings as low as 41 or as high as 42.50 per cent fat.

The individual readings of each person were treated statistically and the values for 3.2 times the probable error of duplicate tests included in Table 1. An examination of these figures shows that most of them range between $\pm 0.45$ per cent, and that the value for all readers on all samples was $\pm 0.444$ per cent. That is to say, it is practically certain that the average of duplicate readings would be between the limits of $41.76 \pm 0.444$ per cent fat.

The results reported by readers 1 and 2, who were more experienced with the test, showed less variation than those reported by the other readers. However, a low degree of variation is not necessarily an indication of more accurate readings. Undoubtedly there was some variation in the actual fat contained in the necks of the test bottles. The reporting of exactly the same value for each test bottle would indicate no variation, but might still involve erroneous reading. Nevertheless, it is logical to assume that the readings reported by the most skilled technicians (1 and 2) more nearly approximate the actual variation of the tests themselves, and that the higher variation reported by the other readers was due to less precision in reading. Readers 1 and 2 were practically certain not to make errors on duplicate readings in excess of $\pm 0.385$ and $\pm 0.390$ per cent from the average test. For readers 3, 4, and 5 any average of duplicate tests within $\pm 0.434$, $\pm 0.483$, and $\pm 0.455$ per cent fat from the mean test might be due to normal variation. Incidentally, it is of interest to note that the limits of variability in reading the tests were correlated with the extent of experience of the readers.

The average of 30 replicate Mojonnier (7) tests on this can of cream was 41.8726, a value which exceeds the average of all Babcock readings by 0.1126 per cent fat.

TABLE 1.—*Limits of normal variation of 456 readings of 96 fat tests on a single can of cream*

| Sample No. | Fat readings, all readers | | | Limits of a practical certainty ± (30 to 1 chance) in reading duplicate fat tests[a] by— | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Minimum | Maximum | Mean | Reader 1 | Reader 2 | Reader 3 | Reader 4 | Reader 5 | All readers |
| | *Per cent* | *Per cent* | *Per cent* | | | | | | |
| 1 | 41.50 | 42.50 | 41.97 | 0.220 | 0.233 | 0.233 | 0.371 | 0.425 | 0.383 |
| 2 | 41.00 | 42.50 | 41.77 | .435 | .348 | .380 | .473 | .288 | .450 |
| 3 | 41.00 | 42.25 | 41.71 | .240 | .326 | .278 | .419 | .480 | .469 |
| 4 | 41.00 | 42.25 | 41.70 | .249 | .381 | .390 | .360 | .700 | .530 |
| 5 | 41.25 | 42.50 | 41.79 | .422 | .390 | .348 | .432 | ---------- | .420 |
| 6 | 41.50 | 42.25 | 41.83 | .348 | .288 | .313 | .300 | ---------- | .339 |
| 7 | 41.25 | 42.25 | 41.73 | .264 | .336 | .361 | .374 | .259 | .360 |
| 8 | 41.25 | 42.00 | 41.60 | .358 | .102 | .240 | .329 | .188 | .298 |
| All samples | 41.00 | 42.50 | 41.76 | .385 | .390 | .434 | .483 | .455 | .444 |

[a] 3.2×probable error of duplicate tests.

### Sweet and Sour Cream

The results obtained from the work reported in Table 1 indicated that a repetition of the experiment with some changes would be advisable. In Table 2 are reported the results of an experiment similar to the one reported in Table 1, except that ten pint samples were taken from a can of sweet cream and 16 Babcock tests were made from each sample. The can of cream was then placed at room temperature until it soured to a thick curdy consistency with an acidity of 0.45 per cent calculated as lactic acid, after which 10 more pint samples were taken and 16 tests made on each sample. Five persons read each test as in the first experiment. There were, therefore, 320 tests on a single can of cream, each read by 5 persons; half of the tests were on sweet cream and half on the same cream after it had soured. The results in Table 2 are based on the 1,599 readings (one broken) from this can of cream.

Table 2.—*Limits of variation of 1,599 readings of 320 tests on cream before and after souring*

| Sample | Sample No. | Fat readings, all readers | | | Limits of a practical certainity ± (30 to 1 chance) in reading duplicate tests * by— | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Minimum | Maximum | Mean | Reader 1 | Reader 2 | Reader 3 | Reader 4 | Reader 6 | All readers |
| | | *Per cent* | *Per cent* | *Per cent* | | | | | | |
| Sweet cream | 1 | 36.00 | 37.50 | 36.98 | 0.364 | 0.230 | 0.313 | 0.454 | 0.643 | 0.442 |
| | 2 | 36.50 | 37.50 | 37.08 | .323 | .352 | .326 | .188 | .336 | .350 |
| | 3 | 36.75 | 37.50 | 37.14 | .259 | .313 | .297 | .291 | .326 | .323 |
| | 4 | 36.25 | 37.50 | 37.10 | .336 | .211 | .380 | .297 | .473 | .382 |
| | 5 | 36.50 | 37.50 | 37.03 | .339 | .198 | .182 | .380 | .553 | .390 |
| | 6 | 36.00 | 37.50 | 36.87 | .352 | .374 | .553 | .313 | .534 | .479 |
| | 7 | 36.50 | 38.00 | 37.14 | .339 | .320 | .534 | .332 | .553 | .437 |
| | 8 | 36.25 | 37.50 | 37.05 | .307 | .000 | .224 | .172 | .470 | .311 |
| | 9 | 36.00 | 37.50 | 36.99 | .371 | .345 | .390 | .358 | .470 | .421 |
| | 10 | 36.25 | 37.50 | 37.02 | .368 | .179 | .294 | .276 | .384 | .384 |
| Same cream after souring | 11 | 36.00 | 37.75 | 37.03 | .518 | .348 | .528 | .508 | .489 | .541 |
| | 12 | 36.00 | 37.50 | 36.94 | .339 | .265 | .310 | .294 | .457 | .403 |
| | 13 | 36.25 | 37.50 | 36.84 | .339 | .460 | .380 | .371 | .396 | .435 |
| | 14 | 36.00 | 37.50 | 36.85 | .361 | .246 | .403 | .083 | .524 | .490 |
| | 15 | 36.25 | 37.25 | 36.83 | .160 | .297 | .348 | .368 | .425 | .370 |
| | 16 | 36.00 | 37.50 | 36.89 | .428 | .320 | .403 | .438 | .409 | .445 |
| | 17 | 36.50 | 37.50 | 36.97 | .361 | .371 | .371 | .339 | .377 | .381 |
| | 18 | 36.00 | 37.50 | 36.84 | .457 | .419 | .409 | .348 | .582 | .454 |
| | 19 | 36.25 | 37.50 | 36.91 | .457 | .422 | .400 | .323 | .489 | .447 |
| | 20 | 36.50 | 37.50 | 36.92 | .227 | .208 | .358 | .204 | .310 | .330 |
| All samples sweet cream | | 36.00 | 38.00 | 37.04 | .356 | .309 | .415 | .338 | .536 | .413 |
| All samples sour cream | | 36.00 | 37.75 | 36.89 | .399 | .382 | .417 | .392 | .491 | .443 |

* 3.2 × probable error of duplicate tests.

Most of the readings on these tests ranged between 36 and 37.50 per cent, a divergence which is comparable to the results reported in Table 1. There were two readings (not tests) reported by one person of 37.75 and 38 per cent, but his readings were not substantiated by the other readers of the same test bottle.

The values for 3.2 times the probable error of duplicate tests confirm the values reported in Table 1. A comparison of the values for sweet cream in Table 2 with those in Table 1 indicates that each reader has reduced slightly the limits of normal variation of his readings. This may be partly due to the experience obtained in the preceding experiment and partly to the fact that the cream had a slightly lower test.

The two parts of the experiment with sweet and sour cream were performed under as nearly identical conditions as possible. The same technician (No. 2) performed the tests, using the same methods and equipment. When the results for sweet and sour cream in Table 2 are compared, it is at once evident that the sweet cream was given a higher test than the same cream after souring. The Mojonnier tests before and after the cream had soured are also noticeably different, being 37.6170 and 37.1125, respectively. The grand averages of the Babcock tests reported by reader No. 2 were $37.050 \pm 0.1365$ and $36.993 \pm 0.1691$ per cent fat for the sweet and sour cream, respectively. The probable error values were based on 160 tests in each case.

The question immediately arises whether the difference of 0.057 is sufficiently large to justify the conclusion that the sour cream contained less fat than the sweet. On calculating, it is found that the probable error of the difference is $\pm 0.2173$. Since the probable error of the difference between the mean tests for sweet and sour cream (0.2173) is nearly four times the actual difference, it is at once evident that the disparity between the means is well within the limits of normal variation.

If the probable error values in Table 2 are used as an index to the relative degree of variability of the tests on sweet and sour cream, it is noted that the values of all but one reader (No. 6) were higher on the sour cream. The greater difficulty of procuring a fair sample on thick curded cream is no doubt responsible for the slightly greater variation evidenced in these results.

### VARIATIONS OF THE TEST IN THE HANDS OF AN EXPERIENCED TECHNICIAN

Reader No. 2 made and read the tests in both experiments (Tables 1 and 2), so that his results are wholly applicable for interpreting the error of the method, whereas the results of the other readers are valuable only as a measure of the error of reading. The probable error of single tests based on his readings in the first experiment was $\pm 0.172$ per cent fat. In reading the 320 tests on sweet and sour cream, normal variation accounted for probable error values of $\pm 0.136$ and $\pm 0.169$, respectively. By calculating the values for 3.2 times the probable error of duplicate determinations, it is found that there is a 30 to 1 chance that the normal variation of his 96 tests reported in Table 1 would not introduce an error of more than 0.390 per cent fat. In other words, he could be practically certain that the average of 2 tests would be within 0.390 of the average of 96 tests. Similarly, from his results on the sweet and the sour cream (Table 2), he could be practically certain that the average of duplicate tests would not be in error more than 0.309 or 0.382 from the result obtained by averaging 160 tests on each.

The application of the probable error of one technician's work to that of another, of course, must be done with reservations. It is evident that one individual may be more or less careful than another, and that the test may yield different results in the hands of an equally experienced operator. However, it is believed that these results do give some tangible evidence of the extent of variation which may be expected when the test is performed by one who has had extensive experience with it. Although the specific decimal figures may not be directly applicable to the work of another individual of equal experi-

ence, the results afford a basis of judging the general quality of work. Any interpretation based on these data must be made with these limitations in mind and with due allowances for them.

### INTERPRETATIVE VALUE OF THE DATA

In the Middle West the large centralized creameries have small cream stations within a radius of 500 miles of the plants. In these cream stations the operators buy, test, and ship the cream, which is brought in by the local dairymen. Even though field superintendents from the plants and representatives from the State dairy commissioner's office frequently check the fat tests of these operators, numerous cases of fraudulent tests are on record. It is in the checking of such fraudulent tests that data such as are presented in this paper may be interpreted to the best advantage.

For the sake of illustration, let it be supposed that a cream station operator had turned in an average of duplicate tests of 40.50 per cent on the same can of cream used in the first experiment. Let it be further supposed that the inspector (reader No. 2) got a test of 41.75 per cent fat on the can of cream. The chances are 30 to 1 that the inspector's average of 2 tests is not more than 0.390 from the "true" test or that the actual fat content of this can of cream is not outside the limits of $41.75 \pm 0.39$ (41.36 and 42.14). Since the station operator's test (40.50) is beyond these limits the inspector is justified in assuming that the station operator is in error. On the other hand, if the station operator's average of duplicate tests were 41.40 per cent, this value, being within the limits of normal variation of the inspector's work, would not be subject to his criticism.

The data in these experiments forcibly illustrate the necessity of making duplicate tests in order to enforce the stipulations of many of the State laws. In Kansas and in several other States, the laws regard any test as fraudulent if it is more than 1 per cent in error. This is interpreted to mean 1 per cent of the fat purchased and not a 1 per cent reading on the neck of the bottle. For example, in buying 100 pounds of cream containing 37 per cent fat, any test beyond the limits of $37 \pm 0.37$ would defraud the buyer or seller of more than 1 per cent of the fat purchased. In other words, the reading of the test in this case must be accurate within 0.37 of the true test to comply with the State law. The results obtained by reader No. 2 with the sour cream, which tested approximately 37 per cent, will be used to illustrate the fact that an inspector could not enforce this stipulation of the law if only single tests were used. The probable error of a single test on this cream was $\pm 0.169$, and 3.2 times this value establishes the limits of certainty at $\pm 0.54$ per cent. In other words, the inspector has demonstrated that normal variation in his own work may account for an error as great as $\pm 0.54$ in the reading of a single test.

If the cream station operator's test on this can of cream were 36.50 and a single test by the inspector were 37, he could not enforce the statutes, even though the disparity between tests exceeded the legal tolerance of 1 per cent of the fat purchased. In other words, the normal variation of the method is likely to exceed the stipulations of the statutes. In fact the data indicate that the normal variation of duplicate samples is just barely within the limits of the 1 per cent

tolerance. Again using the sour cream as an example (Table 2), this inspector has demonstrated that the average of duplicate tests may account for variations as great as ±0.382, which is almost identical with the statute limitations in testing 37 per cent cream. It is evident that in order to comply with a legal tolerance of 1 per cent variation in testing, duplicate determinations must be employed.

### EFFECT OF CARELESS READING

The limits of accuracy of the Babcock method are so near the stipulations of many of the States' laws that precision in every step of the process is requisite. This is well illustrated in the highly variable readings of the tests reported by reader No. 6. (Table 2.) Although this man had had several years experience with the Babcock method and the manipulation of the test constituted one of his daily routine duties, his work was characterized by more speed than accuracy. When the probable error values for reader No. 6 (Table 2) are compared with those of the other readers it is seen that in nearly every case his variations are between wider extremes than are those of the other readers. The limits of variation for the most experienced readers (1 and 2) are just barely within the limits of accuracy demanded by the State laws. The errors resulting from less experience on the part of the other persons (3, 4, and 6) or lack of precision in reading are sufficient to render it doubtful whether they would always comply with the demands of the law.

TABLE 3.—*Distribution of 1,599 readings of 160 tests on sweet cream, and 160 tests on the same cream after souring*

SWEET CREAM

| Reader No. | Number of readings at per cent indicated | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 36 | 36.25 | 36.50 | 36.75 | 37 | 37.25 | 37.50 | 37.75 | 38 |
| 1 | | | 3 | 4 | 93 | 21 | 39 | | |
| 2 | 1 | | 1 | 9 | 120 | 11 | 18 | | |
| 3 | 1 | 1 | 18 | 15 | 86 | 26 | 13 | | |
| 4 | | | 4 | 18 | 76 | 48 | 14 | | |
| 6 | 1 | 6 | 26 | 25 | 63 | 16 | 21 | 1 | 1 |
| Total | 3 | 7 | 52 | 71 | 438 | 122 | 105 | 1 | 1 |

SOUR CREAM

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 16 | 21 | 89 | 20 | 12 | | |
| 2 | | 1 | 19 | 7 | 103 | 14 | 16 | | |
| 3 | 1 | 2 | 45 | 30 | 64 | 16 | 2 | | |
| 4 | 1 | 4 | 10 | 36 | 81 | 22 | 5 | 1 | |
| 6 | 5 | 10 | 55 | 22 | 54 | 9 | 4 | | |
| Total | 8 | 18 | 145 | 116 | 391 | 81 | 39 | 1 | |
| Total for sweet and sour cream | 11 | 25 | 197 | 187 | 829 | 203 | 144 | 2 | 1 |

Table 3 shows the distribution of the 1,599 readings of 160 tests on sweet cream and 160 tests on the same cream after souring. A study of this table shows that most of the extreme readings on both sweet and sour cream were made by one reader (No. 6). The method

of tabulating the individual readings of the tests made it possible to check the work of each reader against that of other readers. In one case, for example, reader No. 6 reported a test of 36 per cent, whereas all the other readers recorded 37 per cent for the same bottle. It is evident in this case that the 36 per cent reading was erroneous and represents a variation due to inaccurate reading rather than to fluctuation in the test. In all cases where the extreme readings of 36, 37.75, and 38 per cent were reported, it was found that these were the result of erratic reading of a single reader, and did not represent the opinion of the other readers.

On examination of the data it was found that all but 2 of the 25 readings of 36.25 per cent were likewise the result of erroneous reading and did not conform to the readings of the majority. In other words, with 2 exceptions, the correct readings of the fat columns of the 320 tests should have been between the extremes of 36.50 and 37.50 per cent. Obviously, in calculating the error of the test all readings whether correct or not must be included, but in interpreting the actual variation of the fat columns in the necks of the bottles, elimination of apparently erroneous readings is justified.

TABLE 4.—*Variations in weighing sixteen 9-gm. samples of cream for the Babcock test*

[All weighings made by the same technician]

| Weight of sample | Weight of sample | Weight of sample |
|---|---|---|
| *Grams* | *Grams* | *Grams* |
| 8.9928 | 8.9774 | 9.0044 |
| 8.9932 | 8.9890 | 8.9747 |
| 8.9998 | 9.0059 | 8.9872 |
| 8.9908 | 8.9641 | 9.0079 |
| 8.9892 | 8.9986 | 8.9939 |
| 8.9988 | | |
| | Mean | 8.9917 |

| | | | |
|---|---|---|---|
| Standard deviation | 0.0112 | Probable error, duplicate weighings | ±.0053 |
| Coefficient of variability | .1245 | 3.2 × Probable error, single weighing | ±.0240 |
| Probable error, single weighing | ±.0075 | 3.2 × Probable error, duplicate weighings | ±.0169 |

## ERROR IN WEIGHING THE SAMPLE

A certain amount of the variation in the results with the Babcock test is undoubtedly due to erroneous weighing. In order to measure the extent of this source of error 16 bottles were carefully weighed on analytical balances before and after admission of the sample. An important aspect of this experiment was that the technician who weighed all the samples was not aware of this check on his work. The results are given in Table 4. The extremes of the weighings of cream were 8.9641 and 9.0079 gm., with a mean of 8.9917 gm. Only 3 of the 16 weighings were in excess of 9 gm. The weighing most closely approximating 9 gm. was 8.9998 or 0.0002, and the most erroneous weighing was 8.9641 or −0.0359 gm. The values for 3.2 times the probable errors for single and duplicate weighings were +0.0240 and +0.0169 gm., respectively. That is to say, the technician in this case could be practically certain of weighing a single sample within +0.0240 gm. of the mean weighing (8.9677 to 9.0157 gm.). Similarly, he could be practically certain that the

average of duplicate weighings would not be more than ±0.0169 gm. from the mean (8.9748 to 9.0086 gm.).

The error of 24 mgm. in weighing 37 per cent cream would cause a variation of 0.1 per cent (0.098) in the reading in the neck of the bottle. Since the limits of practical certainty of testing cream (based on single sample for reader No. 2) were found to be ±0.54 per cent of fat, it follows that about one-fifth of his variation may be traceable to errors in weighing.

### LIMITS OF VARIATION OF THE BABCOCK TEST WHEN UNKNOWN SAMPLES ARE TESTED.

In the experiments reported in Tables 1 and 2 the readers were aware of the fact that the tests were all made from the same can of cream and that the readings, therefore, should be essentially the same. It was recognized that this factor might reduce the error by minimizing the probability of large errors. In order to eliminate this factor, three lots of cream containing approximately 36, 37, and 37.75 per cent fat, respectively, were prepared, and identical samples of each submitted at different times to each of three laboratories. The numbering system employed and the close proximity of the fat tests gave the tester no clue to the identity of the sample being tested, although the technician was aware that his work was being checked. It should be mentioned that these samples were very carefully prepared by the method used in the other experiments, except that approximately 0.03 per cent formaldehyde was added, the screw-cap sample jars dipped in melted paraffin, and the samples kept at 35° to 40° F. until tested.

TABLE 5.—*Frequency distribution of fat test readings on three samples of cream reported by three laboratories*

SAMPLE NO. 1

| Laboratory | Number of readings at per cent indicated | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 35 | 35.5 | 36 | 36.5 | 37 | 37.5 | 38 | 38.5 |
| 1 | | | | 11 | 35 | 3 | 13 | |
| 2 | 4 | 1 | 1 | 12 | 58 | 15 | 1 | |
| 3 | | | | 8 | 80 | | | |
| Total | 4 | 1 | 1 | 31 | 173 | 18 | 14 | |

SAMPLE NO. 2

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | | 6 | 1 | 1 | 1 | | 1 | |
| 2 | 2 | 1 | 8 | 3 | | | | |
| 3 | | 4 | 11 | 3 | | | | |
| Total | 2 | 11 | 20 | 7 | 1 | | 1 | |

SAMPLE NO. 3

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | | | | | 2 | 1 | 5 | |
| 2 | | | | 1 | 2 | 7 | 5 | 3 |
| 3 | | | | | 1 | 1 | 14 | |
| Total | | | | 1 | 5 | 9 | 24 | 3 |

TABLE 6.—*Variation of fat tests on three samples of cream reported by three laboratories*

| Laboratory No. | Tests | Extreme readings | | Average reading | Limits of practical certainty in testing duplicate samples [a] |
|---|---|---|---|---|---|
| | | Low | High | | |
| | *Number* | *Per cent* | *Per cent* | *Per cent* | ± |
| **SAMPLE NO. 1** | | | | | |
| 1 | 62 | 36.5 | 38.0 | 37.14 | 0.74 |
| 2 | 92 | 35.0 | 38.0 | 37.06 | .80 |
| 3 | 88 | 36.5 | 37.0 | 36.95 | .19 |
| Total | 242 | 35.0 | 38.0 | 37.04 | .67 |
| **SAMPLE NO. 2** | | | | | |
| 1 | 10 | 35.5 | 38.0 | 36.05 | 1.25 |
| 2 | 14 | 35.0 | 36.5 | 35.93 | .74 |
| 3 | 18 | 35.5 | 36.5 | 35.97 | .48 |
| Total | 42 | 35.0 | 38.0 | 35.97 | .80 |
| **SAMPLE NO. 3** | | | | | |
| 1 | 8 | 37.0 | 38.0 | 37.68 | .64 |
| 2 | 18 | 36.5 | 38.5 | 37.69 | 1.12 |
| 3 | 16 | 37.0 | 38.0 | 37.90 | .42 |
| Total | 42 | 36.5 | 38.5 | 37.77 | .67 |

[a] 3.2 times probable error of duplicate tests.

Table 5 shows the distribution of the readings reported by the three laboratories on the three samples. In two of the laboratories (Nos. 1 and 2) some difficulty was experienced at first in testing the preserved samples, and although the tests were eliminated until the difficulty was overcome, it may account for part of the variation in the results of these two laboratories. Table 6, based on the data from Table 5, shows the degree of variation of the results to be considerably larger than was reported in Tables 1 and 2. It may be observed that the limits of a practical certainty in making duplicate tests of sample No. 1 were marked by ±0.74, ±0.80, and ±0.19 per cent fat for each of the three laboratories. Although the average readings reported by the three laboratories were not so widely divergent, the extreme readings were very different. The results show that the error of testing may be greater than the preceding experiments would indicate.

TABLE 7.—*Frequency distribution of fat test readings on 44 identical samples of cream* [a]

[Work performed in routine manner at one laboratory]

| Number of readings at per cent indicated | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 32.5 | 36.5 | 37.5 | 37.75 | 38.0 | 38.25 | 38.5 | 39.0 | 41.0 |
| 1 | 1 | 2 | 2 | 23 | 7 | 5 | 2 | 1 |

[a] In this case the extreme low and high readings were 32.5 and 41, respectively, the average reading was 37.97, and the limit of practical certainty in testing duplicate samples (3.2×probable error of duplicate tests) was ±3.23.

As previously mentioned, the technicians were aware that their work was being checked, and, no doubt, may have taken more care in the analyses than regular routine samples would have received. In order to measure the influence of this factor on the error of testing, 44 identical samples were prepared with the same care and precision as

used in mixing samples for the preceding experiments. These samples were sent to one laboratory, a few at a time along with a large number of other routine samples. In this case the tester not only was unaware of the identity of the samples but he was unaware of the fact that he was testing some identical samples in the routine of his day's work. The results reported in Table 7 are surprisingly variable.

It may be observed that the tests varied between the very wide extremes of 32.5 and 41 per cent fat, and that other wide variations were reported. This table also shows the limits of a practical certainty to be 3.23 per cent above or below the mean. Even though the results of this experiment were obtained under practical conditions and from a reputable laboratory, it is difficult to believe that they are representative of widespread conditions. The test of 32.5 per cent might easily have been the result of a 5 per cent error in reading the spread of the dividers on a 37.5 per cent test, but one can hardly explain the misreading of duplicate test bottles on this basis. Similarly, one can easily explain the 41 per cent reading by assuming a slip of the dividers over 3 points on a 38 per cent test, but the reports from this laboratory were supposedly made on the basis of the average of duplicate tests.

Whether or not the results are representative of routine testing, they do show that when check testing was done in the same laboratory (Table 6) much more careful work was reported. In other words, there is a marked tendency to pay less attention to precision in routine procedure than when one is aware that his work is being checked.

## SUMMARY AND CONCLUSIONS

In this series of experiments an attempt was made to measure the limits of error of the Babcock test for cream. A large number of tests were made on a single can of sweet cream, and, in another trial, on cream before and after it had soured. The tests were read by several readers, both experienced and inexperienced. In one experiment identical samples of cream were sent to three laboratories for analysis. In another case samples were sent to one of these laboratories under conditions which prevented the operator from knowing that he was testing check samples.

The results of the first trial, consisting of 456 readings, indicate that the practical limits of variation of the test were 0.444 per cent. The second trial substantiated the results of the first, showing the limits to be 0.413 per cent on sweet cream and 0.443 on sour cream.

The extent of the error depended somewhat on the experience of the reader. However, the tendency of the reader to do precise work was found to be more important than experience.

The distribution of 1,599 readings on a can of sweet cream and the same cream after souring followed the normal curve with 97.5 per cent of the readings falling within approximately 0.5 per cent of the mean. The difference between the results obtained with sweet cream and sour cream was beyond the limits of normal variation of the 160 tests but was within the limits of normal variation when only duplicate tests were employed.

Cream samples which had been weighed into 9-gm. test bottles were reweighed on an analytical balance and found to check very closely to the correct weight. The error due to weighing was responsible for only about one-fifth of the total variation of the test.

The results obtained when submitting samples of known and unknown identity to three laboratories indicate that the error of routine testing is much greater when the operator is unaware that his work is being checked.

In the enforcement of laws pertaining to testing it is important that the limits of error of the test be taken into consideration by the inspector. These data indicate that in most cases an inspector can not be practically certain that a single test will be closer than 0.5 per cent to the correct test. When this normal variation in his own work is ignored in interpreting the disparity between the inspector's and station operator's tests, it may in some instances erroneously show the operator to be a violator of the law. An error of 0.5 per cent on the test on cream containing less than 50 per cent fat would introduce an error in excess of 1 per cent of the amount of fat purchased. This is in excess of the legal tolerance recognized by many States.

The results of these experiments, although not strictly applicable to other workers, show that the technician involved could not be certain that single tests on 37 and 40 per cent cream would be closer than $\pm 0.44$ to $\pm 0.55$ per cent fat, or that the average of duplicate tests would be closer than $\pm 0.31$ to $\pm 0.39$ per cent fat.

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# GENETIC CHARACTERS IN RELATION TO CHROMOSOME NUMBERS IN A WHEAT SPECIES CROSS[1]

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## INTRODUCTION

Combined genetic and cytological studies of wheat species and hybrids between them are used for the information that they give concerning the phylogenetic relationships of the species. They are of interest from the standpoint of plant breeding also, since, in order to attack intelligently a breeding problem by the method of hybridization and selection, it is necessary to know the readiness with which the characters of the parents can be recombined in the progeny and the limitations involved. Species crosses have been used only to a limited extent as a means of obtaining improved varieties of wheat, and only a very few results of practical value have been secured. It is possible that much wider practical use could be made of such crosses if a more thorough knowledge were available concerning their genetic and cytological behavior.

## REVIEW OF LITERATURE

The cultivated species of wheat (*Triticum* L.) on the basis of their chromosome numbers belong to three different groups—the einkorn group (*T. monococcum* L.) with 7 pairs of chromosomes, the emmer group (*T. durum* Desf., *T. polonicum* L., *T. dicoccum* Sch., and *T. turgidum* L.) with 14, and the vulgare group (*T. vulgare* Vill., *T. compactum* Host., and *T. spelta* L.) with 21 pairs. The various cytological results obtained in crosses between different species of the three groups and the amount of sterility in the hybrids have been used as an indication of the probable relationship between the species.

The chromosome behavior in pentaploid hybrids between the emmer and vulgare groups has been described by Kihara (*7*),[3] Sax (*11*), Watkins (*16*), and Thompson (*14*). The hybrid in these crosses receives 14 chromosomes from one parent and 21 from the other. At the metaphase of the first reduction division of the $F_1$, 14 bivalents and 7 univalents are found, the 14 emmer chromosomes having paired presumably with 14 from the vulgare parent. The bivalents behave

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[3] Reference is made by number (italic) to Literature Cited, p. 178.

normally in both divisions. The univalents lag until the members of the bivalents have left the equatorial plate, then split equationally and go to the poles. In the second division, the univalents go to the poles at random. Some irregularities are found, such as the loss of univalents or the failure of univalents to split at the first division. On this basis, gametes formed by the $F_1$ would vary in the number of chromosomes from 14 to 21, 16, 17, and 18 being probably most frequent. Watkins (*17*) studied meiosis in the megaspore mother cells and came to the conclusion that the same course is followed in the reduction divisions of both the megaspore and microspore mother cells, the loss of chromosomes occurring about as frequently in one as in the other.

In the crosses of emmer and vulgare wheats, Sax (*12*, *13*) found a striking association between chromosome numbers and morphological characters. A large proportion of the $F_2$ and $F_3$ segregates resemble one parent or the other, a preponderance resembling the emmer parent. In $F_2$ and following generations, the only fertile segregates are those having 14 chromosome pairs and resembling the emmer parent, or those with 21 pairs and resembling the vulgare parent. Some of the fertile segregates, however, may have one or two characters that are typical of the other type, but there is no evidence that the typical and desirable characters of the parents have ever been combined in a single variety of economic value.

The desirable characters include resistance to black stem rust, *Puccinia graminis tritici* Eriks. and Henn., and apparently Sax believes that it is quite improbable that a variety of the vulgare type could be obtained from such a cross with the rust resistance of the durum parent.

The report of Thompson (*14*) shows that the correlation between chromosome numbers and morphological characters is not so great as that suggested by Sax. In a cross of *Triticum vulgare* × *T. durum*, he studied the inheritance of resistance to stem rust and 13 pairs of characters which distinguish *T. durum* from *T. vulgare*. His results agree with those of Sax except in a few instances. He obtained more intermediates in the $F_2$ and $F_3$, although these tended to disappear in the $F_3$ and later generations. The plants with 14 pairs of chromosomes were of the durum type, and those with 21 pairs were of the vulgare type, but most plants of each type showed a few characters of the other type. A few vulgare plants were found resistant to stem rust, but to a lesser degree of resistance than that found in the durum parent. It would seem from this that any desired recombination of characters could be obtained.

In studies conducted with spring wheat using the genetic mode of attack, Hayes et al. (*5*) found that in crosses between a durum resistant to stem rust and a vulgare susceptible to it there was an indication of linkage of the durum characters and resistance. They show, however, that if large enough numbers are used, it is quite possible to get the desired combination of durum and vulgare characters. From one of these crosses between Marquis (*Triticum vulgare*) and Iumillo (*T. durum*) a fertile homozygous variety of wheat of the vulgare type has been obtained. This variety, Marquillo, a brief description of which is given by Hayes and Garber (*4*), has 21 pairs of chromosomes, is about equal to the vulgare parent in milling and baking qualities,

and has at least two genetic factors for stem-rust resistance which it inherited from its durum parent (*6*). It is susceptible to root rot, another character obtained from the durum parent. Hayes and Garber (*4*) also report some important results which have been obtained by McFadden in South Dakota. From a cross of Emmer (*T. dicoccum*) and Marquis, several wheats of the vulgare type highly resistant to stem rust were produced. Hope, one of these varieties, has not been tested sufficiently to determine whether it will be very valuable as a commercial variety. As stated by Clark and Ausemus (*3*) the immunity of Hope wheat from black stem rust and other diseases can be transmitted readily to selections from crosses with other vulgare varieties.

A. A. Sapehin (*9*) confirms the facts obtained by Hayes et al. In crosses between *Triticum vulgare* and *T. durum*, he found, as usual, a very complicated segregation of genetic characters. He obtained a large number of recombinations of the characters of both parents. Three of the vulgare types were resistant to Hessian fly, a character which they inherited from the durum parent. A most interesting type of plant obtained in this study from the cytological standpoint contained 36 chromosomes (16 bivalents and 4 univalents). This remained constant in chromosome number and morphological characters for a period of six years. This seems all the more remarkable since Kihara considered this a sterile combination. L. A. Sapehin (*10*) gives a report of the durum segregates of crosses of *T. durum* and *T. vulgare*. A cytological examination shows balanced sets of 28 chromosomes in these segregates, but the presence of so many vulgare characters indicates that, though the parental number has been retained, the sets differ qualitatively. In this work again is mentioned segregates resistant to Hessian fly and to drought.

In view of the seemingly conflicting results which have been obtained by different investigators, it is evident that much more work must be done with species crosses before the problem is solved entirely.

## MATERIAL AND METHODS

The investigation reported in this paper was undertaken as a combined genetic and cytological problem to determine if possible the relationships between chromosome numbers and genetic characters in a cross between Velvet Don (*Triticum durum*) and Quality (*T. vulgare* Host.) and to throw more light on the feasibility of using such crosses in breeding improved varieties of wheat.

The cross was made in 1925, and the genetic and cytological studies of the $F_1$, $F_2$, and $F_3$ were carried on during the years 1926, 1927, and 1928, respectively. Cytological material of about 100 $F_2$ plants was examined, but definite chromosome counts and genetic results were obtained for only 24 of them. About 500 $F_3$ plants were examined in the same manner, results being secured for 68 of these.

Velvet Don has 14 pairs of chromosomes; a prominent keel extending the full length of the outer glume; a prominent collar which is a structure extending completely around most of the stems at the base of the lowest spikelet; a solid stem in the internode just below the spike; long, narrow glumes; a rachis internode length of about 3.6 mm.; long awns; heavily pubescent glumes; and red seed. It is highly resistant under field conditions to stem rust (*Puccinia graminis tritici*) and to leaf rust (*P. triticina* Eriks.).

Quality, on the other hand, has 21 pair of chromosomes; a less pronounced keel extending only part of the length of the glume; collar absent or extending only part way around the stem; hollow stem; shorter and wider glumes than are found in Velvet Don; an internode length of about 6 mm., tip awns, glabrous glumes, and white seed. It is susceptible to both stem and leaf rust in the field.

Characters such as chromosome number, keel form, collar, stem cavity, glume shape, and internode length, as contrasted in Velvet Don and Quality, distinguish nearly all durum from vulgare varieties; the other characters are common to both species.

The seed for the $F_1$ of this cross was turned over to the writer by Dr. H. K. Hayes of the Minnesota Agricultural Experiment Station. The $F_1$ plants were grown in an isolated plot to prevent crossing with other wheats. The $F_3$ lines were grown in the plant pathology nursery at University Farm, St. Paul, Minn., where they were subjected to an artificial epidemic of stem rust. A heavy leaf rust also was obtained on the $F_3$ lines, although quite by accident. Evidently the spray used to induce the stem-rust epidemic carried leaf-rust spores as well. Rust notes were taken on individual plants in the field. Notes on other plant characters were determined on a single head of each individual plant in the laboratory. To obtain the rachis internode length, 10 internodes, beginning with the second from the base, were measured in millimeters. The average of these 10 was taken as the index of internode length. The percentage of seed set as an indication of sterility was taken on the $F_1$ plants by counting the total number of heads in a line, obtaining the average number of spikelets per head and the total number of seeds produced. Two seeds per spikelet were used as 100 per cent fertility, and the percentage of seed set was calculated on that basis. In the $F_3$ data, the plants were placed in five groups on the basis of their fertility. The plants in Group 1 were completely fertile, those of Group 2 about 75 per cent fertile, Group 3 about 50 per cent, Group 4 about 25 per cent, while Group 5 was completely sterile. A few plants were designated 5− to indicate almost complete sterility.

It was part of the plan to make a study of the rust reactions in the seedling stage of the segregates of each of the generations in the greenhouse. As a preliminary to such a study, the parents, Velvet Don and Quality, were tested in the greenhouse in the winter of 1925–26. Although Velvet Don has been known to be highly resistant to stem rust in the field for 20 years, it was found to be very susceptible in the seedling stage in these tests. Both Velvet Don and Quality were inoculated with 14 different physiologic forms of stem rust. Velvet Don was found to be susceptible to all of them, but Quality showed some resistance to forms Nos. 10, 19, 23, 27, and 38. (Table 1.)

TABLE 1.—*The reaction to stem rust of wheat, Puccinia graminis tritici, of Velvet Don and Quality in the seedling stage in the greenhouse*

[In this table, the symbol R stands for resistant, MR moderately resistant, MS moderately susceptible, and S susceptible]

| Parent | Reaction to rust form No. — | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 10 | 15 | 18 | 19 | 21 | 23 | 27 | 29 | 33 | 36 | 38 | 40 | 41 |
| Velvet Don | S | S | S | S | S | S | S | S | S | S | S | S | S | S |
| Quality | S | MR | S | S | R | S | MR | MR | S | MS | S | MR | S | S |

From these tests, it was evident that no segregation could be expected in reaction to many of the physiologic forms in the seedling stage in the greenhouse, since both of the parents were susceptible to many of the forms. The rust tests were made, therefore, in the field, and in order that the readings might be comparable all plantings were made on the same date. This was a disadvantage from the standpoint of the cytological studies, since it gave only a short period of time in which good material could be obtained.

Chromosome counts were made in the meiotic divisions of the pollen mother cells. Belling's (*2*) iron-acetocarmine method was used to examine the anthers in order to obtain the right stages. The anthers found to be in the proper stage of development were fixed in Allen's (*1*) modified Bouin's solution. The usual method of making permanent slides for such material was followed. The sections were cut from 10$\mu$ to 15$\mu$ thick, those with a thickness of 12.5$\mu$ giving the best results. A few of the slides were stained with Haidenhain's iron-alum haematoxylin, but for most of them a modified triple stain combination was used. By this method, a nice contrast was obtained between the chromatin material and the cytoplasm by staining in a 1 per cent aqueous solution of safranin for one hour or more. If chromosome counts only are required, the gentian violet and orange G of the triple stain may be omitted, which is an advantage from the standpoint of time and expense.

Chromosome counts were made in the metaphase or early anaphase of the first division. The number of bivalents and univalents were obtained, and in most cases the number of univalents were checked in the side view of the metaphase.

## GENETIC AND CYTOLOGIC DATA

Only a few chromosome counts were made of the parents of the Velvet Don × Quality cross, but these were enough to verify the fact that Velvet Don cytologically was a typical durum with 14 pairs of chromosomes behaving regularly, and Quality was a vulgare with 21 pairs. The characters of each of the parents have been described in Material and Methods.

The $F_1$, as has been reported before for such crosses, has a total of 21 chromosomes in the metaphase of the first division—14 bivalents and 7 univalents. This was verified by the total of 35 found in the early anaphase of the first division before the univalents had split. The $F_1$ plants had a keel and collar similar to the durum, although there were frequent variations resulting in an intermediate condition for these characters. The stem cavity and glume shape were intermediate between the parents. The pubescence and red seed color of the Velvet Don were dominant. The beards were intermediate. The $F_1$ plants were not artificially inoculated to obtain an epidemic of stem rust, but a trace to 5 per cent of rust was observed, the shape and size of the pustules clearly indicating susceptibility.

The $F_1$ plants were spaced about 6 inches apart in the field, to give an opportunity for tillering. One hundred and forty-seven plants were grown, and these produced 1,278 heads, or on the average about 8.7 heads per plant. Fifteen hundred and eighty-four seeds were harvested. On the basis that there were on the average

12.5 spikelets per spike, and that two seeds per spikelet constitute perfect seed setting, the $F_1$ plants set seed to the extent of about 5 per cent.

The chromosome counts and genetic results obtained for the parents, $F_1$ and $F_2$ are given in Table 2.

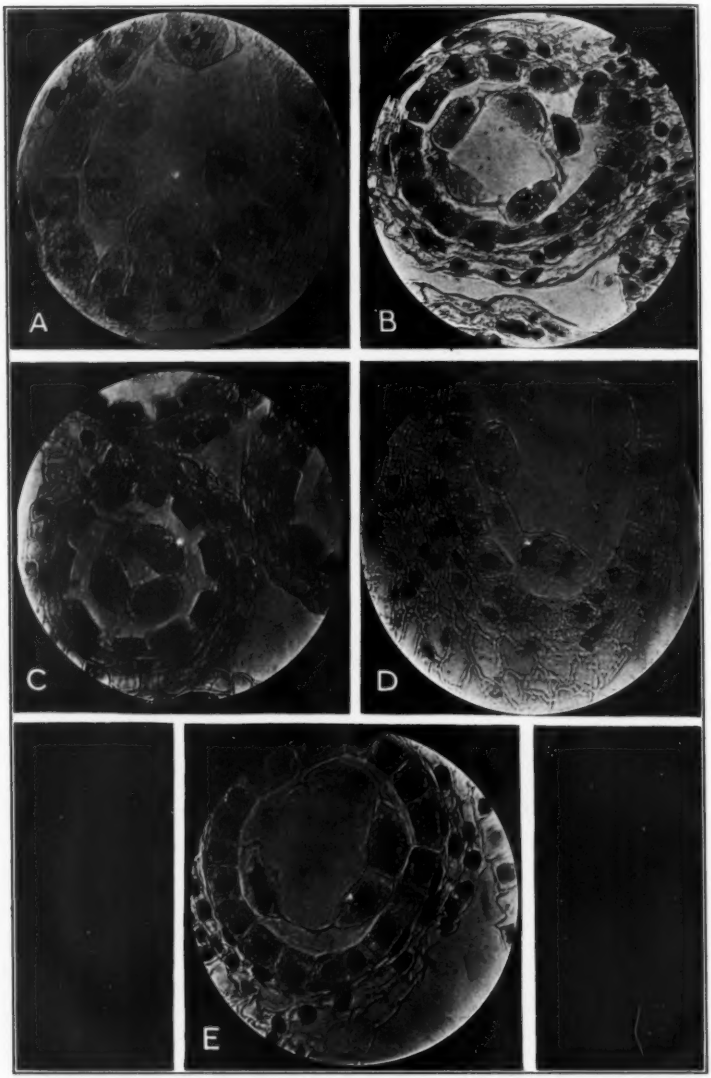
TABLE 2.—*Chromosome numbers and characters of parents, $F_1$ and $F_2$ plants of the Velvet Don×Quality wheat cross* [a]

| Plant No. | Somatic No. | Metaphase No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color |
|---|---|---|---|---|---|---|---|---|---|
| Velvet Don $P_1$ | 28 | $14_{II}$ | D | D | D | D | b | P | R |
| Quality $P_1$ | 42 | $21_{II}$ | V | V | V | V | B | p | r |
| $F_1$ | 35 | $14_{II}$–$7_I$ | D | D | I | I | I | P | R |
| $F_2$: | | | | | | | | | |
| 567–3 | 42 | $21_{II}$ | D | D | V | D | B | p | R |
| 567–6 | 38 | $17_{II}$–$4_I$ | D | V | V | V | B | P | R |
| 567–7 | 28 | $14_{II}$ | D | V | I | V | I | P | r |
| 567–8 | 29 | $14_{II}$–$1_I$ | D | D | D | D | B | p | R |
| 569–1 | | | V | D | V | V | I | P | R |
| 569–2 | 42 | $21_{II}$ | D | D | V | D | I | p | R |
| 570–5 | 42 | $21_{II}$ | D | V | I | D | I | P | R |
| 571–1 | | | V | V | V | V | B | P | r |
| 571–5 | 30 | $14_{II}$–$2_I$ | D | D | V | D | I | P | R |
| 571–6 | 29 | $14_{II}$–$1_I$ | D | D | D | I | B | p | R |
| 571–9 | 42 | $21_{II}$ | D | D | I | I | b | P | R |
| 571–12 | 30 | $14_{II}$–$2_I$ | D | D | D | D | B | P | R |
| 571–14 | 29 | $14_{II}$–$1_I$ | D | D | D | D | I | P | R |
| 572–4 | 28 | $14_{II}$ | D | D | D | D | I | p | r |
| 572–12 | 28 | $14_{II}$ | D | D | I | D | I | P | r |
| 572–14 | 42 | $21_{II}$ | D | D | V | D | B | P | R |
| 574–5 | 32 | $15_{II}$–$2_I$ | D | D | D | D | B | P | r |
| 575–5 | 28 | $14_{II}$ | V | V | D | V | B | p | r |
| 576–2 | 28 | $14_{II}$ | D | D | D | D | I | P | R |
| 577–3 | 28 | $14_{II}$ | D | D | D | V | b | p | R |
| 577–4 | 28 | $14_{II}$ | D | D | D | I | I | p | R |
| 579–4 | 28 | $14_{II}$ | D | D | V | D | B | P | R |
| 580–4 | 35 | $14_{II}$–$7_I$ | D | D | V | I | B | P | R |
| 582–6 | 28 | $14_{II}$ | D | D | D | D | I | P | R |
| 583–3 | 28 | $14_{II}$ | D | D | D | D | B | P | r |
| 583–7 | 28 | $14_{II}$ | V | V | V | V | b | p | r |

[a] Symbols used in tables: $_{II}$, bivalents; $_I$, univalents; D, durumlike; V, vulgarelike; I, intermediate; B, beardless or tip awned as the vulgare parent; b, bearded; P, pubescent; p, glabrous; R, red; r, white.

Five $F_2$ plants had 42 chromosomes as the somatic number. One had 38; 1, 35; 1, 32; 2, 30; 3, 29; and 11, 28.

Three of the 42-chromosome plants had as many as 3 of the 4 differentiating characters—keel, collar, stem cavity, and glume shape—inherited from the durum parent. The other 2 plants of this group had 2 such characters. The 38-chromosome plant had a durum keel, but vulgare collar, stem cavity, and glume shape. The plant with 35 chromosomes had a durum keel and collar, with a vulgare stem cavity and an intermediate glume shape. The plant with 32 chromosomes was durumlike in the four differentiating characters. Plant 571–5 with 30 chromosomes was durumlike in keel, collar, and glume shape, but had a vulgarelike stem cavity, while the other 30-chromosome plant, 571–12, had all four of these characters durumlike. Two of the three 29-chromosome plants were durumlike in all 4 of these characters, but the other one had durum keel, collar, and stem cavity, with a vulgare glume shape. The 28-chromosome plants showed a somewhat greater association between chromosome number and surum characters than did those with 42. Four of the 28-chromodome group had all 4 of the differentiating characters durumlike,




PHOTOMICROGRAPHS OF POLLEN MOTHER CELLS IN MEIOTIC DIVISION

A, Metaphase side view of the first division of the $F_3$ plant No. 7–1 showing two lagging univalents, one on each side of the plate; B, metaphase polar view of the first division of the $F_2$ plant 32–14 showing 14 bivalents; C, metaphase polar view of the first division of another $F_3$ plant showing 14 bivalents on the equatorial plate; D, anaphase side view of the completely sterile $F_3$ plant 21–35 which had 39 chromosomes, 18 bivalents, and 3 univalents; the photomicrograph shows 3 unpaired univalents just after they have split equationally (same cell as is shown in Fig. 1, G); E, anaphase of the first division of a 14-chromosome plant showing the characteristic shape of the chromosomes.

4 others had 3 such characters, 2 had 1, and 1 was vulgarelike for all 4 of the characters—keel, collar, stem cavity, and glume shape.

On the basis of chromosome numbers, it is seen that the results obtained are similar to what has been described by other workers for such crosses; that is, more 28's and 42's and fewer intermediates than would be expected if gametes with from 14 to 21 chromosomes were formed in the theoretical ratio, and a random mating of these gametes took place in the production of viable zygotes.

In a number of the $F_2$ plants, frequent end-to-end pairing of some of the bivalents was observed. Trisomes and tetrasomes were also found.

Cytological material of a large number of plants of the $F_3$ lines was examined, and notes were taken on the morphological character of each one. Chromosome counts and genetic characters were obtained on 68 plants, and this report will include only the results from these. The progeny of the 42-chromosome plants were studied first, as the vulgare type is of more interest to the plant breeder. The $F_3$ line No. 7 is the progeny of a 42-chromosome plant. The data on chromosome numbers and genetic characters are given in Table 3.

TABLE 3.—*Chromosome numbers and characters in an $F_2$ plant (567–3) and the $F_3$ line derived from it*

| Plant No. | Somatic No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *Mm.* | |
| $F_2$ 567–3 | 42 | D | D | V | D | b | p | R | | | | |
| $F_3$ | | | | | | | | | | | | |
| 7–1 | 42 | V | V | V | V | b | p | R | 80S | [a] 25S | 7.5 | [b] 2 |
| 7–5 | 42 | D | D | V | D | b | p | R | 85S | T | 7.0 | 1 |
| 7–12 | 42 | V | V | V | V | I | p | R | 60S | 5R | 7.1 | 1 |
| 7–15 | 42 | D | V | V | V | b | p | R | 80S | 30S | 8.0 | 2 |
| 7–16 | 42 | D | D | V | D | b | p | R | 70S | 5R | 6.8 | 3 |
| 7–17 | 42 | V | V | V | V | b | p | R | 80S | 5R | 8.2 | 2 |
| 7–27 | 42 | D | D | V | D | b | p | r | 80S | 10R | 7.4 | 3 |
| 7–28 | 42 | D | D | V | D | b | p | r | 80S | 5R | 5.1 | 1 |
| 7–32 | 42 | V | V | V | V | b | p | R | 80S | 10S | 6.8 | 1 |
| 7–34 | 42 | D | D | I | D | b | p | R | 65S | 20S | 7.5 | 2 |
| 7–39 | 42 | D | I | V | V | b | r | p | 80S | 30S | 7.1 | 1 |
| 7–40 | 42 | D | V | V | V | b | p | r | 60S | 30S | 6.4 | 1 |
| 7–41 | 42 | V | V | V | D | b | p | R | 80S | 20S | 6.1 | 1 |
| Velvet Don check | 28 | D | D | D | D | b | P | R | 7R | 2R | 3.6 | 1 |
| Quality check | 42 | V | V | V | V | B | p | r | 81S | 71S | 6.0 | 1 |

[a] Symbols used—With reference to leaf or stem rust: T, trace; R, resistant; SR, semiresistant; S, susceptible. The numbers give the percentage of rust found. For other symbols used see footnote *a*, Table 2.

[b] Numbers indicate degrees of sterility as follows: 1, completely fertile; 2, about 75 per cent fertile; 3, about 50 per cent fertile; 4, about 25 per cent fertile; 5—, almost completely sterile; 5, completely sterile.

Thirteen plants of this line were all in the 42-chromosome group. This would indicate that the line is uniform for chromosome number and might be expected to remain constant in later generations. Univalents were found occasionally, but not more frequently than in the vulgare parent. A photomicrograph of a pollen mother cell of plant No. 7–1 (pl. 1, A) shows the bivalents grouped on the equatorial plate with two univalents, one on either side of the plate.

With only 13 plants to choose from, the data show some interesting combinations of durum and vulgare characters. The $F_2$ parent plant resembled Velvet Don in keel, collar, glume shape, beards, and seed color, and Quality in stem cavity and pubescence. Segregation, as shown by the $F_3$ line, took place for keel, collar, glume

shape, and seed color. Twelve of the plants had hollow stem cavities such as are found in the vulgare parent. One was classified intermediate, but this may have been a vulgare also with slightly thicker culm walls than is usually found in the vulgare types. The $F_2$ parent plant was bearded and should have bred true for this character. One $F_3$ plant, 7–12, was classified as intermediate for beards, but this may have been caused by field hybridization. All the plants of the line studied were susceptible to leaf rust, but their reaction to stem rust varied from very resistant to susceptible, although the highest percentage of stem rust found on them was less than half of that found on Quality, the susceptible $P_1$. The internode lengths of all these plants were comparable to that of the vulgare parent. No very high correlation seemed to exist between the 42-chromosome number and the vulgare characters, although considered as a whole the vulgare characters outnumbered the durum. Some association between stem-rust resistance and distinctive durum characters is seen in the fact that four of the plants highly resistant to stem rust had three typical durum characters each. Despite this, No. 7–17 is a plant in which is combined the chromosome number and characters of the vulgare parent and the stem-rust resistance of the durum $P_1$. This plant was very fertile, showing about 75 per cent seed setting. Plant 7–12 is another vulgare type which combines the stem-rust resistance and part of the leaf-rust resistance of the durum with the typical vulgare characters. It is especially interesting from the standpoint of plant breeding that, in a very small number of plants, such combinations can be obtained. If a progeny test in later generations shows these to be constant for chromosome numbers and homozygous for botanical characters, they represent recombinations which the plant breeder would consider desirable.

Lines 12 and 13 are the progeny of the $F_2$ plant 569–1. The data for chromosome numbers and characters of these lines are given in Table 4.

TABLE 4.—*Characters of an $F_2$ plant (569–1) and chromosomes and characters of the $F_3$ line derived from it* [a]

| Plant No. | Somatic No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *Mm.* | |
| $F_2$: 569–1 | | V | D | V | V | I | p | r | | | | |
| $F_3$: 12–1 | 42 | V | V | V | V | B | p | r | 85S | 60S | 5.3 | 2 |
| 13–1 | 42 | V | V | I | V | I | p | R | 5R | 60S | 5.7 | 3 |
| 13–4 | 42 | V | D | V | V | I | p | R | 85S | 70S | 6.8 | 1 |
| 13–6 | 42 | V | V | V | V | B | p | r | 85S | 70S | 6.3 | 3 |
| 13–9 | 42 | V | V | V | I | I | p | R | 70S | 15R | 6.5 | 2 |
| 13–12 | 42 | V | V | V | V | I | p | R | 70S | 70S | 5.6 | 2 |
| 13–20 | 42 | V | D | V | V | B | p | r | 80S | 40S | 7.0 | 1 |
| 13–21 | 42 | V | D | D | I | I | p | r | 70S | 50S | 4.4 | 3 |
| 13–23 | 42 | V | D | V | V | B | p | r | 85S | 5R | 6.4 | 4 |
| 13–27 | 42 | V | V | V | V | B | p | R | 80S | 5R | 4.6 | 2 |
| 13–29 | 42 | V | V | V | V | B | p | r | 85S | 15SR | 6.7 | 2 |
| 13–32 | 42 | V | V | V | V | I | p | R | 75S | 60S | 6.0 | 3 |
| Velvet Don check | 28 | D | D | D | D | b | P | R | 7R | 2R | 3.6 | 1 |
| Quality check | 42 | V | V | V | V | B | p | r | 81S | 71S | 6.0 | 1 |

[a] For explanation of symbols see footnote *a*, Table 2, and footnotes *a* and *b*, Table 3.

No reliable chromosome count was obtained on 569–1, but it had the appearance of a vulgare. The $F_3$ data show that it was probably a plant having 21 pairs of chromosomes, since all 12 $F_3$ plants were found to be in that class. From the $F_3$ data the $F_2$ parent should have been classified as red for seed color since a segregation occurred, and from the $F_1$ that red seed color was dominant. This is an error which is easily made in classifying the seed of species hybrids for color, because the shriveled condition of the seeds of some plants makes such classification very difficult. The results here emphasize again the importance of a progeny test to verify an $F_2$ classification. Three of the four characters differentiating the two species were of the vulgare type in the $F_2$ parent of lines 12 and 13. As a consequence, the $F_3$ plants were uniform for the vulgare type of keel, stem cavity, and glume shape. One of the plants, 13–21, was classified as durum for stem cavity, but irregularities of this nature occasionally occur.

The segregates of lines 12 and 13 varied in their reaction to stem rust from 5R to 70S. From this $F_3$ line, it would be possible to obtain again vulgarelike segregates with 42 chromosomes and the stem rust resistance of the durum $P_1$. In no case so far discussed has resistance to both stem and leaf rust been found in the same plant. Plant No. 13–1, however, is vulgarelike in most of its characters and is as resistant to leaf rust as Velvet Don. A progeny test of this plant will have to be made to determine the constancy for the different characters, especially for its leaf rust reaction.

The $F_3$ line No. 9 was variable for all the characters studied except seed color. Table 5 gives the data for chromosome numbers and genetic characters for this line.

TABLE 5.—*Chromosome numbers and characters in an $F_2$ plant (567–6) and the $F_3$ line derived from it*

| Plant No. | Somatic No. | Metaphase No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | *Mm.* | |
| $F_2$: 567–6 | 38 | $17_{II}$–$4_I$ | D | V | V | V | B | P | R | | | | |
| $F_3$: | | | | | | | | | | | | | |
| 9–19 | 36 | $16_{II}$–$4_I$ | D | D | V | V | B | p | R | 50S | T | 4.8 | 2 |
| 9–26 | 38 | $17_{II}$–$4_I$ | D | D | I | V | I | P | R | 80S | 20SR | 5.0 | 4 |
| 9–28 | 33 | $15_{II}$–$3_I$ | D | D | V | D | B | P | R | 60S | 20S | 5.4 | 3 |
| 9–32 | 34 | $15_{II}$–$4_I$ | D | V | V | V | I | P | R | 40S | 60S | 5.3 | 5— |
| 9–38 | 42 | $21_{II}$ | V | V | V | V | B | p | R | 80S | 70S | 5.3 | 2 |

[a] For explanation of symbols see footnote *a*, Table 2, and footnotes *a* and *b*, Table 3.

The $F_2$ plant No. 567–6, from which this line was grown, had approximately 38 chromosomes. From two to six univalents were observed in side views of the metaphase of the first division. The behavior of the chromosomes was very irregular. Many end-to-and bivalents were observed as well as many trisomes. Other irregular combinations were frequent. A polar view of a pollen mother cell in the metaphase of the first division is shown in Figure 1, A. The $F_3$ plants, 9–19, 9–26, 9–28, and 9–32, were as difficult to study as the parent, and the counts in all these are only approximate. Plants 9–19 and 9–28 were quite similar to the parent in chromosome behavior Trisomes occurred frequently and occasionally a tetrasome was found.

Plant 9–32 with 34 chromosomes was almost completely sterile, only two or three shriveled seeds being found in it. A polar view of an anaphase of the first division of this plant is shown in Figure 1 B.

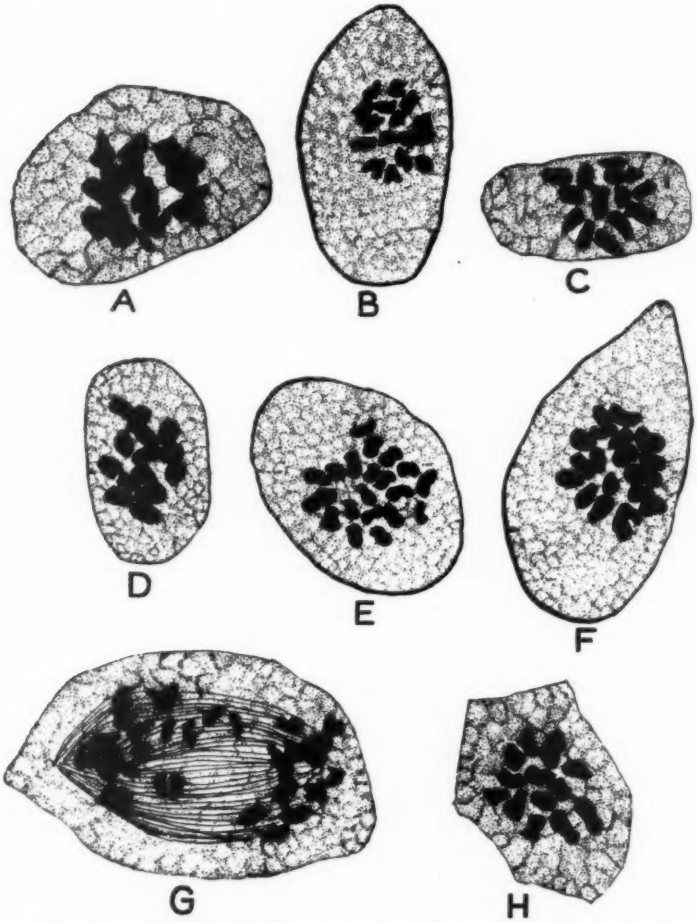



FIGURE 1.—Pollen mother cells in meiotic division. The figures were drawn from single sections, a camera lucida being used: A, Metaphase polar view of the first division of the $F_2$ plant 567–6 showing about 17 bivalents and 4 univalents. B, Anaphase polar view in the $F_2$ plant 9–32 which had 34 chromosomes, the drawing showing one group of 17. C, Metaphase polar view of the first division of the $F_2$ plant 571–14 which had 14 bivalents and 1 univalent. D, Metaphase polar view of the $F_3$ plant 34–14 which had 14 bivalents and was almost completely sterile. E, Metaphase polar view of the first division of the $F_3$ plant 7–28 showing 21 bivalents; this plant had the stem-rust resistance of the durum parent. F, Metaphase polar view of the first division of the $F_2$ plant 13–1 showing 21 bivalents; this plant had the leaf-rust resistance of the durum parent. G, Anaphase side view of the completely sterile $F_3$ plant 21–35 which had 39 chromosomes with 18 bivalents and 3 univalents; the drawing shows 3 unpaired univalents just after they have split equationally. H, Metaphase polar view of the first division of the $F_3$ plant 56–18 showing 14 bivalents; this plant had the stem-rust susceptibility of the vulgare parent

The data for the $F_2$ plant 571–1 and its $F_3$ progeny are given in Table 6.

TABLE 6.—*Characters of an $F_2$ plant (571–1) and chromosomes and characters of the $F_3$ line derived from it* [a]

| Plant No. | Somatic No. | Metaphase No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | *Mm.* | |
| $F_2$: 571–1 | | | V | V | V | V | B | P | r | | | | |
| $F_3$: | | | | | | | | | | | | | |
| 21–5 | 29 | $14_{II}$–$1_I$ | V | V | D | V | B | P | r | 20SR–S | 15S | 4.4 | 2 |
| 21–7 | 28 | $14_{II}$ | V | D | D | D | B | P | R | 10R | T | 3.5 | 2 |
| 21–22 | 28 | $14_{II}$ | D | D | D | D | I | P | R | 70S | 40S | 4.0 | 3 |
| 21–23 | 30 | $14_{II}$–$2_I$ | V | D | D | D | B | P | R | 10R | 5R | 3.5 | 4 |
| 21–25 | 42 | $21_{II}$ | V | D | V | V | B | P | R | 75S | 40S | 4.6 | 4 |
| 21–35 | 39 | $18_{II}$–$3_I$ | D | D | D | D | B | P | | | T | 5.3 | 5 |
| 21–36 | 35 | $14_{II}$–$7_I$ | D | V | I | V | I | P | R | 80S | 50S | 5.3 | 3 |
| 21–40 | 28 | $14_{II}$ | D | D | D | D | B | P | R | 20R–SR | T | 3.8 | 3 |

[a] For explanation of symbols used see footnote *a*, Table 2 and footnotes *a* and *b*, Table 3.

The $F_2$ parent of line 21 was classified as vulgare, but a reliable chromosome count was not obtained. The $F_3$ results lead to the conclusion that it had a high chromosome count, either 42 or approaching 42 in number. A large $F_3$ line was raised from it, showing that it was a fairly fertile combination. The segregations for genetic characters as found in the $F_3$ line are difficult to account for. There is the possibility that the $F_2$ was incorrectly classified for several of its characters. It seems certain, however, that the characters were more vulgare-like than durum. One plant, No. 21–35, is of special interest. It contained 39 chromosomes. The univalents varied from three to seven. Three univalents in the process of division are shown in Figure 1, G. Plate 1, D, is a photomicrograph of the same pollen mother cell. Although this plant belonged in the higher group from the standpoint of chromosome numbers, it had the durum type of character for keel, collar, stem cavity, glume shape, and stem-rust resistance. It was, however, completely sterile and is thus eliminated from further tests.

The breeding behavior of a plant which, from counts made in the anaphase of the first division, had a total of 30 chromosomes is shown in Table 7.

TABLE 7.—*Chromosome numbers and characters in an $F_2$ plant (571–12) and the $F_3$ line derived from it* [a]

| Plant No. | Somatic No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *Mm.* | |
| $F_2$: 571–12 | 30 | D | D | D | D | B | P | R | | | | |
| $F_3$: | | | | | | | | | | | | |
| 32–6 | 28 | D | D | D | D | B | P | R | 5R | T | 3.4 | 3 |
| 32–9 | 28 | D | D | D | D | B | p | R | 5R | 60S | 3.3 | 4 |
| 32–14 | 28 | D | D | D | D | B | P | R | 5R | 60S | 2.8 | 2 |
| 32–16 | 28 | D | D | D | D | b | P | r | 5R | 30S | 3.3 | 4 |
| 32–17 | 28 | D | D | D | D | B | P | R | 5S | 20S | 3.8 | 3 |
| 32 19 | 28 | D | D | D | D | B | P | R | 5R | T | 4.1 | 2 |
| 32–23 | 28 | D | D | D | D | B | P | R | 5R | T | 3.8 | 3 |
| 32–34 | 28 | D | D | D | D | B | P | r | 5R | 60S | 3.8 | 1 |
| 32–35 | 28 | D | D | D | D | b | p | R | 5R | 10S | 3.7 | 3 |
| 32–39 | 28 | D | D | D | D | B | P | r | 5R | 5R | 4.3 | 3 |

[a] For explanation of symbols see footnote *a*, Table 2 and footnotes *a* and *b*, Table 3.

All of the plants of the $F_3$ line 32 had 14 pairs of chromosomes in the metaphase of the first division. This indicates that the gametes with 14 chromosomes produced by this parent plant were either more numerous or more effective in the fertilization process. Slight irregularities in pairing were found in these plants, but on the average they were similar to the durum parent. The $F_2$ parent was durum-like from the standpoint of its morphological characters of keel, collar, stem cavity, and glume shape. All the plants of the $F_3$ line were of the same type for these four characters. The internode length also showed them to belong to the durum class. Segregation occurred for beards, pubescence, and seed color. Nine of the ten plants gave a leaf rust reaction of 5R, the other one of 5S. The reaction for stem rust varied from a trace to 60S, showing a range from the resistance of the Velvet Don to almost the susceptibility of the Quality. The association of chromosome numbers and durum characters with stem-rust resistance is not, apparently, very great in this line, since there were three plants nearly as susceptible as the vulgare $P_1$, and only four with the resistance of the durum $P_1$. In this case, the susceptibility of the vulgare parent has been found in plants with the chromosome number and differentiating morphological characters of the durum parent.

The data for chromosome numbers and characters of a 29-chromosome plant, 571–14, and its $F_3$ progeny are given in Table 8.

TABLE 8.—*Chromosome numbers and characters in an $F_2$ plant (571–14) and the $F_3$ line derived from it* [a]

| Plant No. | Somatic No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *Mm.* | |
| $F_2$: 571–14 | 29 | D | D | D | D | I | P | R | | | | |
| $F_3$: | | | | | | | | | | | | |
| 34–1 | 28 | D | D | D | D | B | P | R | 10SR–S | 30SR | 3.5 | 2 |
| 34–2 | 28 | D | D | D | D | B | p | r | 10S | 50S | 3.1 | 5– |
| 34–4 | 28 | D | D | D | D | B | P | R | 5SR | 5R | 3.4 | 3 |
| 34–5 | 28 | D | D | D | D | B | P | R | 15SR | | 3.5 | 5– |
| 34–6 | 28 | D | D | D | D | I | P | R | 15SR | 20SR | 3.4 | 5– |
| 34–7 | 28 | D | D | D | D | B | P | R | 20R–S | 5R | 3.2 | 3 |
| 34–8 | 28 | D | D | D | D | B | P | R | 20S | 50S | 3.2 | 2 |
| 34–9 | 28 | D | D | D | D | B | P | R | 30S | 5R | 3.8 | 2 |
| 34–12 | 28 | D | D | D | D | B | P | R | 20SR–S | 60S | 3.5 | 3 |
| 34–14 | 28 | D | D | D | D | B | p | R | 15SR | 10SR | 2.7 | 5– |
| 34–15 | 28 | D | D | D | D | b | p | | 20SR | 30S | 3.1 | 5 |
| 34–16 | 28 | D | D | D | D | B | p | R | 20SR | 60S | 3.8 | 2 |

[a] For explanation of symbols see footnote *a*, Table 2, and footnotes *a* and *b*, Table 3.

The $F_2$ plant, 571–14, had 29 chromosomes. The pairing in this plant was very irregular. Tetrasomes were found frequently. A pollen mother cell in the metaphase of the first division with 15 chromosomes is shown in Figure 1, C. This plant bred true for keel, collar, stem cavity, and glume shape. From the standpoint of the differentiating characters and internode length, all 12 plants in this line would be classified as durum. These plants all have intermediate types of reaction to leaf rust. Although there is variation, none of them shows the resistance to leaf rust of the durum parent. About the same variation is found in the reaction to stem rust of the various plants of this line as was found in line 32, with little association of stem-rust resistance and durum characters.

The most interesting feature of line 34 is the amount of sterility found in some of the plants. One plant showed complete sterility and four others were almost completely sterile, producing only a very few shriveled seeds. This is a good illustration of the importance of chromosome quality rather than numbers, and the result of incompatability of chromosomes which show enough affinity for each other, even to form pairs. Lines 32 and 34 are the progeny of plants which Kihara (7) designated as belonging to fertile combinations of the "decreasing group." The plants of this group decrease in chromosome numbers in later generations until the somatic number 28 of the durum $P_1$ is reached. These two lines and their parents furnish additional evidence as proof of this hypothesis.

Two $F_3$ lines were grown from plants having 14 pairs of chromosomes and classified as durums for the characters of the keel, collar, stem cavity, and glume shape. Chromosome counts and genetic data were obtained on eight of the $F_3$ plants. (Table 9.)

TABLE 9.—*Chromosome numbers and characters in two $F_2$ plants (572–4 and 576–2) and the $F_3$ lines derived from them* [a]

| Plant No. | Somatic No. | Keel | Collar | Stem cavity | Glume shape | Beards | Pubescence | Seed color | Leaf rust | Stem rust | Internode length | Sterility |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | *Mm.* | |
| $F_2$: 572–4 | 28 | D | D | D | D | I | p | r | | | | |
| $F_3$: | | | | | | | | | | | | |
| 38–1 | 28 | D | D | D | D | B | P | R | 25SR | 10SR | 4.0 | 3 |
| 38–7 | 28 | D | D | D | D | B | P | R | 5R | T | 3.6 | 2 |
| 38–8 | 28 | D | D | D | D | B | p | R | 10S | T | 4.4 | 3 |
| $F_2$: 576–2 | 28 | D | D | D | D | I | P | R | | | | |
| $F_3$: | | | | | | | | | | | | |
| 56–2 | 28 | D | D | D | D | b | P | r | 5R | 40S | 3.2 | 2 |
| 56–5 | [b] 28 | | | | | | | | | | | |
| 56–6 | 28 | D | D | D | D | B | P | R | 10SR | 40S | 3.2 | 2 |
| 56–18 | 28 | D | D | D | D | B | P | R | 5R | 60S | 4.0 | 3 |
| 56–20 | 28 | D | D | D | D | B | P | r | 5R | 20SR | 3.4 | 2 |
| 56–27 | 28 | D | D | D | D | B | P | R | 20R–8R | 30S | 3.1 | 4 |

[a] For explanation of symbols see footnote *a*, Table 2, and footnotes *a* and *b*, Table 3.
[b] No head matured.

All of these plants had 14 pairs of chromosomes, and all had the characteristic durum type of keel, collar, stem cavity, glume shape, and internode length. As a result, all of them would be classified as durum plants. The $F_2$ plant, 572–4, may have been classified incorrectly for pubescence and seed color as indicated by its breeding in the $F_3$. There is the possibility that three $F_3$ plants resulted from natural crosses with a 14-chromosome male parent. All eight of the plants of these two lines showed an intermediate amount of fertility, only one of them, 56–27, showing as low as 25 per cent seed setting. Four out of the eight plants showed the Velvet Don reaction to leaf rust, but none of them was as susceptible as Quality, although some were intermediate to the parents.

The reaction to stem rust varied from 5R, which is similar to Velvet Don, to 60S, which is nearly as susceptible as Quality. Such a wide variation found in so few plants does not suggest a very close association between chromosome number, typical durum characters, and manner of reaction to leaf and to stem rust.

The primary purpose of this study was to determine, if possible, the relationships between chromosome numbers and genetic characters in a cross between a durum variety of wheat with 14 pairs of chromosomes and a vulgare variety with 21 pairs. The phase of the work upon which most emphasis was placed and which seemed to be the most important from the standpoint of the plant breeder of the spring-wheat section was to determine if the stem-rust resistance of the durum variety could be obtained in vulgare segregates of this cross. As a consequence, a study of all the cytological phases of the problem was not attempted. A few of the cytological features that have more or less direct relationship to the problem are of sufficient interest to be recounted here.

The parents were studied to determine if they were typical of the species to which each belonged from the standpoint of chromosome numbers. The $F_1$ was not studied in all its phases, but the metaphase and anaphase of the first division showed that the cytological behavior was similar to that described by other workers for pentaploid hybrids. Several of the $F_2$ plants were very irregular in their chromosome behavior. Plant 567–6 had approximately 38 chromosomes. From two to six univalents were observed in side views of the metaphase of the first division. Many end-to-end bivalents were observed as well as many trisomes. Other irregular combinations were frequent. A polar view of a pollen mother cell in the metaphase of the first division is shown in Figure 1, A. The $F_3$ progeny of this plant varied in chromosome numbers, which is direct genetical proof that the $F_2$ parent did not have 21 pairs of chromosomes. Several of the plants of the $F_3$ line derived from this $F_2$ were as difficult to study as the parent. One of them, No. 9–32, is recorded as a 34-chromosome plant in Table 5, and a drawing of the polar view of the early anaphase of the first division is given in Figure 1, B. The univalents, as shown by side views of the metaphase of the first division, varied from none to four.

The $F_2$ plant, 571–14, the progeny of which was grown in the $F_3$ had 29 chromosomes. The pairing in this plant was very irregular. Several tetrasomes were found. A pollen mother cell in the metaphase of the first division with 15 chromosomes is shown in Figure 1, C. All the $F_3$ plants of this line had 14 pairs of chromosomes, which would indicate that the greater number of gametes formed by the $F_2$ parent had 14 chromosomes or that the gametes with 14 chromosomes were more effective in the fertilization process. One of the most interesting plants of this line (Table 8) is No. 34–14 which was almost completely sterile. The metaphase of the first division of this plant is shown in Figure 1, D. As far as can be determined, there seems nothing abnormal about the chromosomes here observed, so that an explanation of the high degree of sterility in plants of this type can not be based on chromosome number.

The interesting $F_3$ segregates from the standpoint of the main purpose of this study are those with 21 pairs of chromosomes and stem or leaf rust resistance. Plant No. 7–28 (Table 3) has 21 pairs of chromosomes and is the equal of the durum parent in resistance to stem rust. The metaphase of the first division, showing 21 chromosomes, is given in Figure 1, E. The $F_3$ plant, No. 13–1, also has 21 pairs of chromosomes and is as resistant to leaf rust as Velvet Don. A pollen mother cell showing the metaphase of the first division with

21 chromosomes is illustrated in Figure 1, F. Univalents are sometimes found in these plants, but no more frequently than in the vulgare parent. A photomicrograph of a pollen mother cell of plant No. 7–1 shows two lagging univalents. (Pl. 1, A.)

Plant 21–35 showed 21 chromosomes at the metaphase of the first division—18 bivalents and 3 univalents. An anaphase of this plant is shown in Figure 1, G. The three univalents appear in this figure just after they have split equationally. This illustrates the usual behavior of unpaired univalents in plants in which bivalents and univalents are found together. A photomicrograph of the same cell is shown in Plate 1, D. This plant was completely sterile.

Another recombination which was obtained from this cross was the 14-chromosome number of the durum parent and the vulgare susceptibility to stem rust. This type is illustrated by plant No. 56–18, the chromosomes of which are shown in Figure 1, H. Another plant, No. 32–14, shows this same combination. A photomicrograph of the metaphase of the first meiotic division of the latter plant is given in Plate 1, E.

An illustration of a polar view of the metaphase of the first division of another 14-chromosome plant is shown in Plate 1, C. In this, the chromosomes are separated more widely on the equatorial plate than was the case in Plate 1, B. Accurate counts can often be made in the early anaphase of the first division. (Pl. 1, E.) Counts of both groups should be made, however, before the total number in the cells can be determined. This is true especially in plants with unpaired univalents. The two parts of the cell are found often in adjacent sections of the slide. If the members of the bivalents are in the condition in which they can be counted, the univalents are still lagging and have not split equationally. They may be found then in one or the other of the parts of the cell. This frequently would give different numbers in the two sections. As an example, a 35-chromosome plant gave 19 and 16, 17 and 18, and 15 and 20 in counts of this kind. Such counts might be misleading from the standpoint of the behavior of unpaired univalents, since it might appear that they go to the poles by random in the first division.

A side view of the later anaphases of such plants frequently shows, however, the lagging unpaired univalents scattered around the equatorial plate in more or less advanced stages of equational splitting. This would indicate that they split at the first division.

## DISCUSSION

The chromosome behavior in the cross, Velvet Don × Quality, is similar to that reported by other workers for pentaploid hybrids. When bivalents and univalents are found in the same plant, there is evidence that the bivalents behave normally in both divisions, but that the univalents lag, come to the plate later in the first division, split equationally, and join the members of the bivalents at the poles.

The fact that there are fewer plants among the $F_2$ segregates with an intermediate number of chromosomes is explained by Sax (*13*) as due to the elimination of gametes with intermediate numbers of chromosomes, because they are less fertile and less viable than those with either 14 or 21 chromosomes. The absence of a complete set of chromosomes results in gametic sterility, and the absence of a com-

plete diploid set results in sterility in the zygote. Thompson and Cameron (*15*) suggest that the plants with intermediate chromosome numbers occur less frequently in $F_2$ and certain $F_3$ lines, not only because of gametic elimination, but also because of zygotic mortality. Kihara (*7*) divided the possible combinations into fertile and sterile groups on the basis of the number of bivalents and univalents which they possessed. Many of the intermediate types are given as fertile combinations. As evidence that the number of bivalents and univalents in the combination is not always a reliable index of sterility, the work of A. A. Sapehin (*9*) may be cited again. He obtained a 36-chromosome plant having 16 bivalents and 4 univalents which remained constant in morphological characters and chromosome number for six years. A combination with the same number of bivalents and univalents was given by Kihara as sterile.

Further evidence that combinations of certain numbers of bivalents and univalents do not always determine the degree of sterility has been obtained in the Velvet Don × Quality cross reported in this paper. One of the $F_3$ segregates, No. 21–35 (Table 6), had 18 bivalents and 3 univalents but was completely sterile. This combination was reported by Kihara as fertile. In this cross, too, one $F_3$ segregate with 14 pairs of chromosomes was completly sterile, while three others were almost in the same condition. These plants have a well-balanced condition as far as chromosome numbers are concerned. It is not reasonable to suppose, however, that the chromosomes were all derived from the original durum parent, since, although the plants have durumlike morphological characters, they show complete or very high degree of sterility. They must, therefore, represent a combination of durum and vulgare chromosomes that is out of balance from the standpoint of genetic factors. This condition must have resulted because of an exchange of chromosomes or parts of chromosomes which are different in quality between the two parent species.

Sax (*13*) concludes that the 14 emmer chromosomes are essentially similar to the 14 vulgare chromosomes with which they pair, that the 7 extra vulgare chromosomes determine most of the typical characters of this group of wheat species, and that the desirable and typical characters of the emmer and vulgare wheats can seldom if ever be combined. The cytologic and genetic data obtained from the cross, Velvet Don × Quality, show that recombinations between the typical characters of the durum and vulgare parents occur with relative frequency. Some association between chromosome numbers and morphological characters is apparent, but relatively homozygous fertile segregates in which are combined the valuable characters of both species occur in sufficient numbers to make feasible the use of such a cross in plant breeding. Several plants were found in the $F_3$ which combined vulgare characters and chromosome numbers with the stem-rust resistance of the durum parent. The leaf-rust resistance of Velvet Don was found in one segregate of the vulgare type and chromosome number. Several plants with 14 chromosomes and morphological characters of the durum had the stem rust susceptibility of the vulgare parent. Both stem and leaf rust resistance were combined in some of the 14-chromosome durumlike segregates, but in no case was resistance to these two diseases present in the same 21-chromosome vulgare plant. To find a plant of the latter type

resistant to leaf rust and another one of the same type resistant to stem rust is a step in advance, however, since a cross between these two probably would give the desired recombination without the difficulties encountered in a species cross.

The amount of seed setting in the $F_1$ of this cross, 5 per cent, makes it possible to get a large $F_2$ population with a reasonable amount of work. Sterility in the $F_2$ from whatever cause, eliminates a number of segregates, but some of the fertile ones should give the desired results. If apparently homozygous fertile segregates combining the valuable characters of the vulgare and durum parents are found in the relatively small numbers which can be used in a cytologic study, it is reasonable to expect that almost any combination can be obtained when large numbers are used, as is done in a comprehensive genetic study. Evidence of the same nature is given by Thompson (*14*) from results obtained in a combined genetic and cytologic study. He found that, while there is a rather definite association between chromosome number and other characters in each of the species, the correlation between stem-rust resistance and durum characters had been broken, although he found no vulgarelike plants as resistant as the durum parent. The important breeding results which have been obtained by Hayes et al. in the production of Marquillo wheat, by McFadden in the production of Hope wheat, by A. A. Sapehin in the production of vulgare strains of wheat resistant to the attacks of Hessian fly and to drought, and by Nina Meister (*8*) who reported the production of drought-resistant and winter-resistant strains of wheat from wheat-rye crosses, all point to the fact that species hybrids can be used as an important means of obtaining varieties of value from an economic standpoint.

The facts obtained in all the studies enumerated seem to be contradictory to the hypothesis stated by Sax. It is true that the cytologic and genetic information is still fragmentary and not entirely conclusive, and that much more work will have to be done before all the questions of sterility and of chromosome and genetic behavior can be answered in detail. Enough is known already, however, to assure the plant breeder that if sufficiently large numbers are used there is every reason to believe that any desired recombination of the characters of the parents of a species cross can be secured.

## SUMMARY

The relationships between chromosome numbers, characters of keel, collar, stem cavity, glume shape, reaction to stem rust, and reaction to leaf rust were studied in segregates of a pentaploid hybrid, *Triticum durum*, variety Velvet Don, × *T. vulgare*, variety Quality.

The $F_1$ had a total of 21 chromosomes in the metaphase of the first division, 14 bivalents and 7 univalents. The $F_1$ plants had the durum type of keel and collar, an intermediate condition for stem cavity and glume shape, intermediate condition for beards, and the pubescence and red seed color similar to the durum parent. They were susceptible to stem rust. They set seed to the extent of about 5 per cent.

The chromosome behavior in this cross was for the most part similar to what was been reported for such hybrids by other investigators. When bivalents and unpaired univalents are found in the same plant, the bivalents behave normally in both meiotic divisions. The univalent

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ents lag, come to the plate late, divide equationally, and join the members of the bivalents at the poles.

Association between the chromosome number of each species and the typical characters of the same is apparent, but recombinations of the characters of both species are relatively frequent.

Relatively homozygous, fertile $F_3$ segregates with the vulgare chromosome number, keel, collar, stem cavity, and glume shape combined with the stem-rust resistance of the durum have been obtained. One fertile vulgarelike $F_3$ segregate had the leaf-rust resistance of the durum.

Durumlike plants with 14 chromosome pairs were found that were as susceptible to stem rust as the vulgare parent. All the plants of one $F_3$ line had 14 pairs of chromosomes and durumlike characters, but with an intermediate type of resistance to leaf rust, although they varied somewhat in this respect.

One $F_3$ plant with 14 pairs of chromosomes was completely sterile, and three others almost sterile, giving only a very few shriveled seeds. One $F_3$ plant with 39 chromosomes was completely sterile. Another with 34 chromosomes gave only a few shriveled seeds.

The relative frequency with which recombinations of the characters of the two species occurred is evidence that a cross of this kind can be used to advantage in plant breeding and is contrary to the conclusion that the desirable and typical characters of the emmer and vulgare wheats can seldom, if ever, be recombined.

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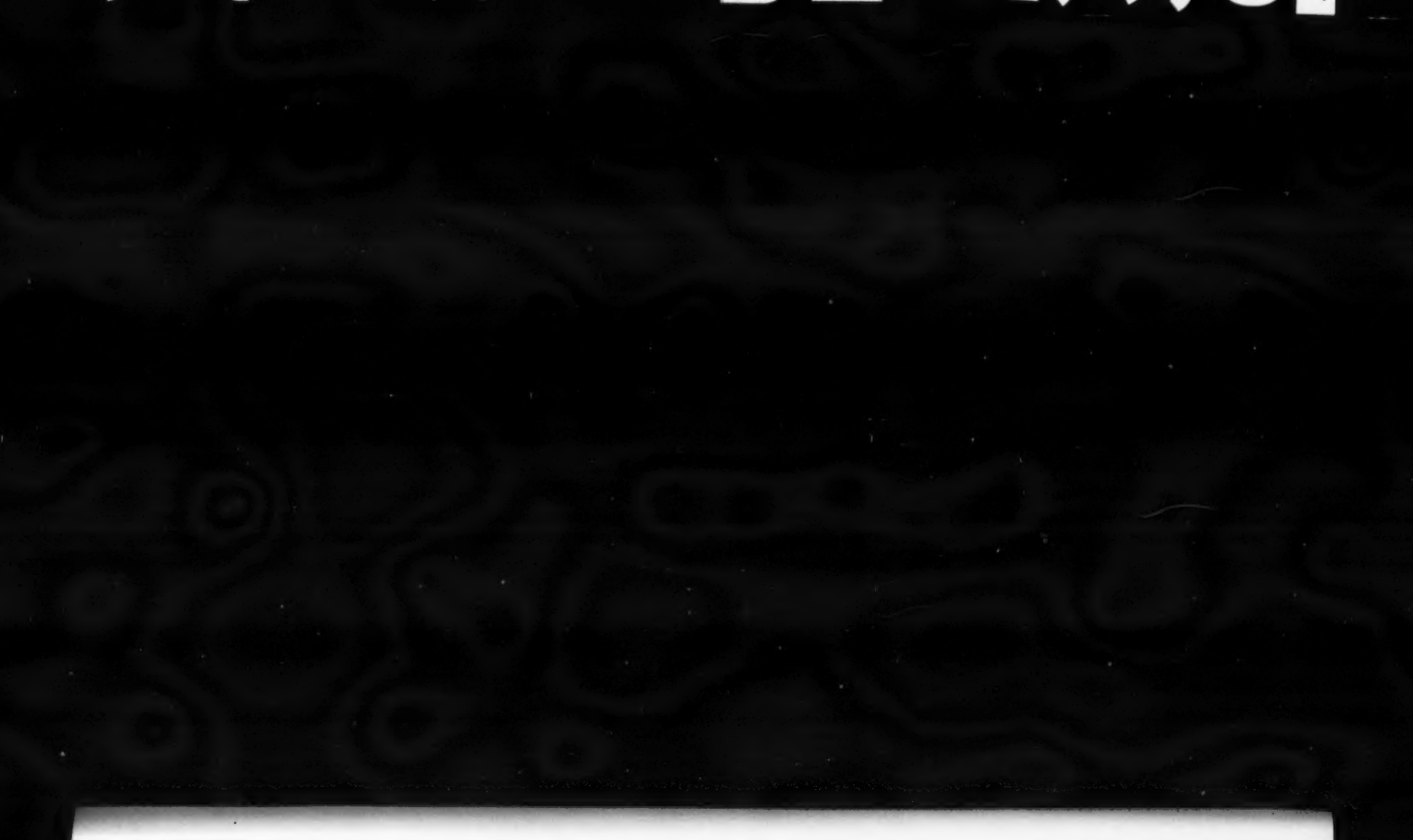
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